

Automated Oligosaccharide Synthesis

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ETH

Eidgenössische Technische Hochschule Zürich
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BURNHAM INSTITUTE
for MEDICAL RESEARCH

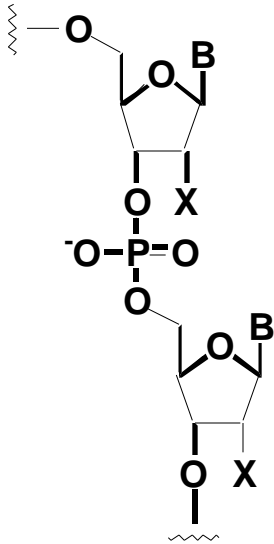
From Research, the Power to Cure

Biopolymers: Overview

Genomics

Proteomics

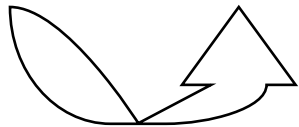
Glycomics



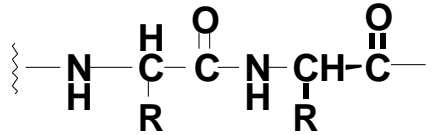
Transcription

Translation

Replication

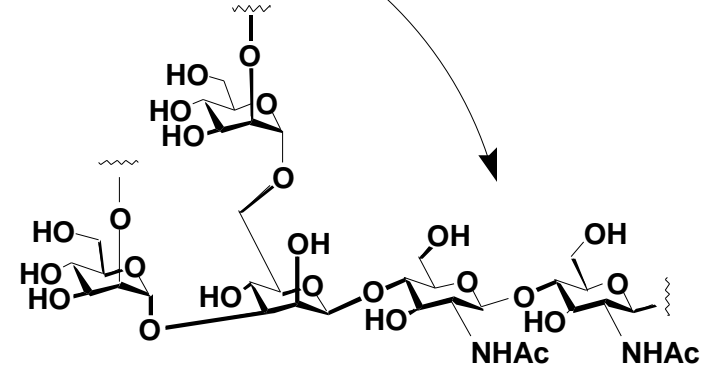


Nucleic Acids



Proteins

Glycosyltransferases

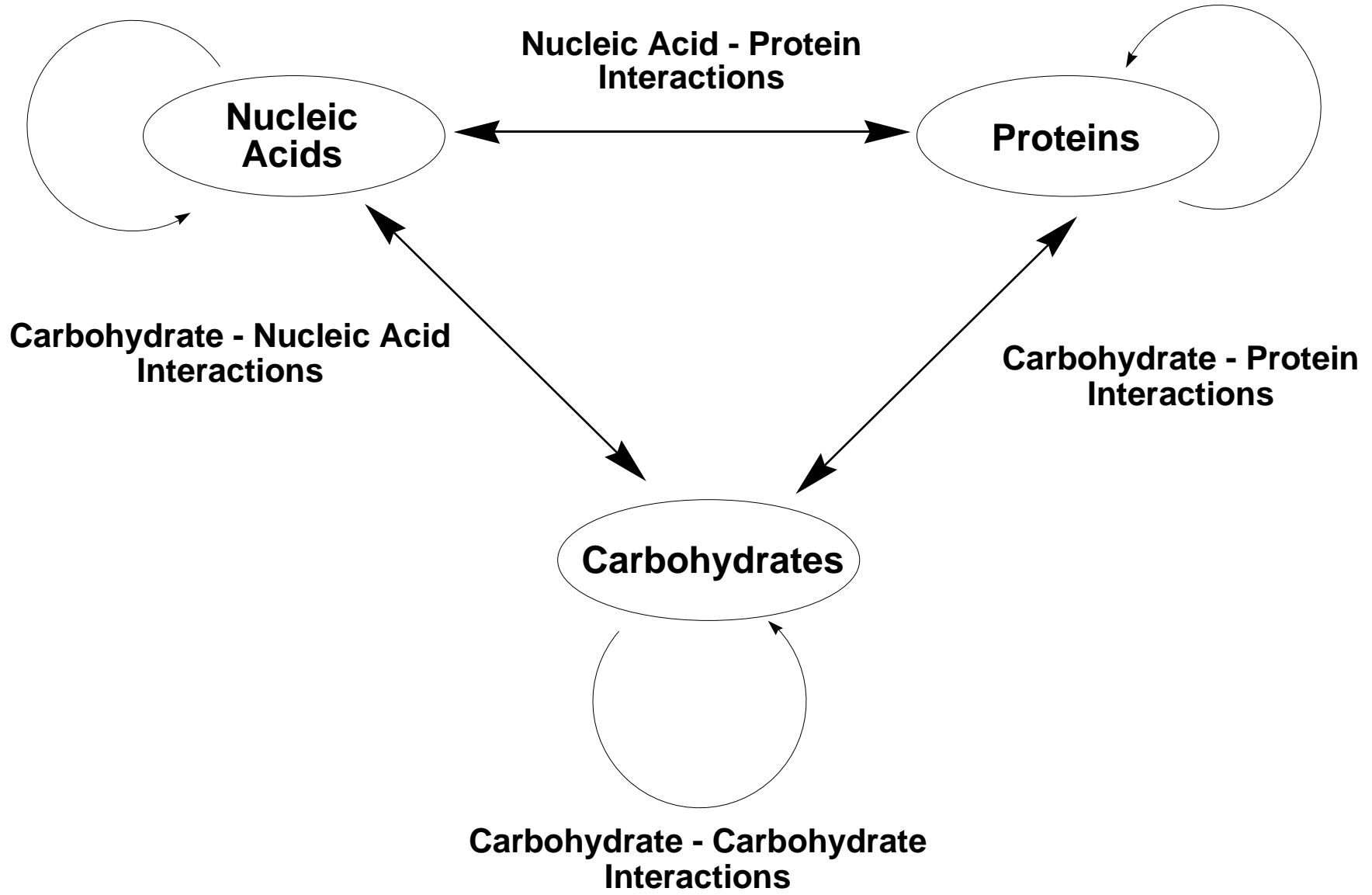


Oligosaccharides - Glycoconjugates

Biopolymer Interactions

Nucleic Acid - Nucleic Acid Interactions

Protein - Protein Interactions



Nucleic Acid - Protein Interactions

Nucleic Acids

Proteins

Carbohydrate - Nucleic Acid Interactions

Carbohydrate - Protein Interactions

Carbohydrates

Carbohydrate - Carbohydrate Interactions

Studies on Polynucleotides†

CIII.‡ Total Synthesis of the Structural Gene for an Alanine Transfer Ribonucleic Acid from Yeast

H. G. KHORANA^a, K. L. AGARWAL^a, H. BÜCHI^b, M. H. CARUTHERS^a,
N. K. GUPTA^c, K. KLEPPE^d, A. KUMAR^c, E. OHTSUKA^f,
U. L. RAJBHANDARY^a, J. H. VAN DE SANDE^a, V. SGARAMELLA^e,
T. TERAOKA^b, H. WEBER^g AND T. YAMADA^h

*Institute for Enzyme Research of the University of Wisconsin and the
Departments of Biology and Chemistry, Massachusetts Institute of
Technology, Cambridge, Mass. 02139, U.S.A.*

(Received 9 December 1971)

A plan for the total synthesis of the DNA duplex, 77 nucleotide units long, corresponding in sequence to the major yeast alanine transfer RNA, is formulated. The plan involves: (a) the chemical synthesis of 15 polydeoxynucleotide segments ranging in length from five to 20 nucleotide units and (b) ligase-catalyzed covalent joining of several segments to form three parts of the duplex, followed by joining of the three parts to construct the entire duplex. Twelve accompanying papers describe the experimental realization of this objective.

Comment by Cell Biology Correspondent

“...This is perhaps the greatest tour de force organic and biochemists have yet achieved. Like NASA with its Apollo program, Khorana’s group has shown it can be done, and both feats may never be repeated...”

Nature **1973**, 241, 33.

Synthesis of Deoxyoligonucleotides on a Polymer Support¹

M. D. Matteucci and M. H. Caruthers*

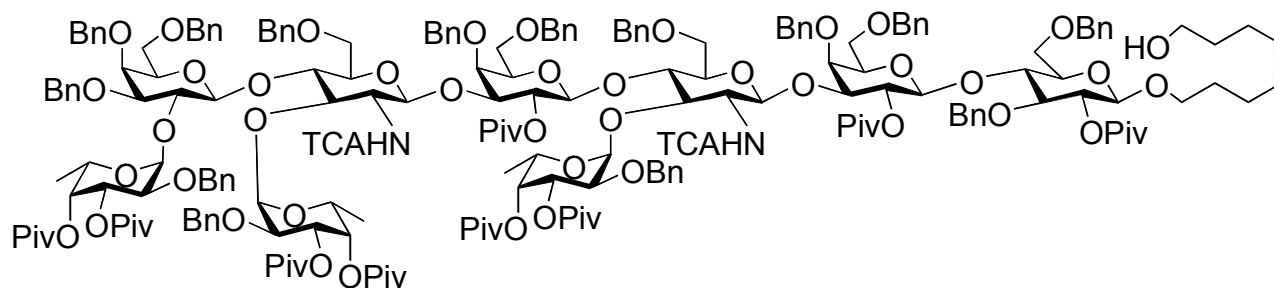
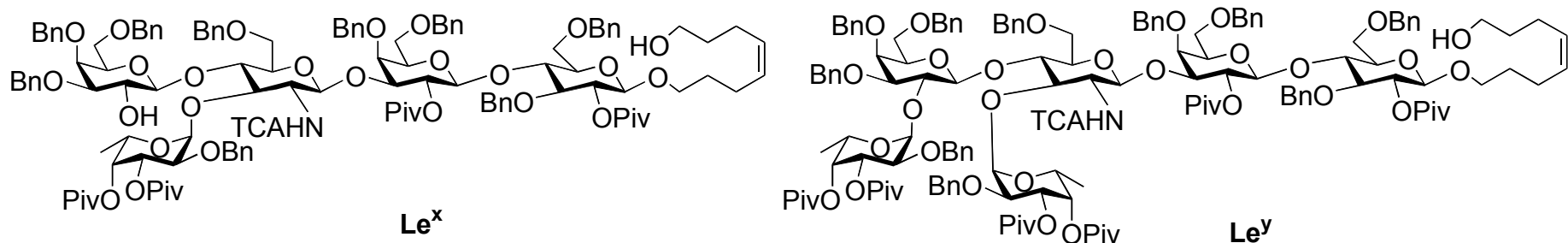
*Contribution from the Department of Chemistry, University of Colorado,
Boulder, Colorado 80309. Received September 18, 1980*

Abstract: The development of a new method for synthesizing deoxyoligonucleotides is described. The synthesis begins by derivatizing high-performance liquid chromatography grade silica gel to contain 5'-O-(dimethoxytrityl)deoxynucleosides linked through the 3'-hydroxyl to a carboxylic acid functional group on the support. This matrix is then packed into a column which is attached to a pump and a series of valves. The chemical steps for the addition of one nucleotide to the support are as follows: (1) detritylation using $ZnBr_2$ in nitromethane (30 min); (2) condensation of a 5'-O-(dimethoxytrityl)deoxynucleoside (3'-methoxytetrazoyl)phosphine with the support-bound nucleoside (60 min); (3) blocking unreacted, support-bound nucleoside hydroxyl groups with diethoxytriazolylphosphine (5 min); (4) oxidation of phosphites to phosphates with I_2 (5 min). Completed deoxyoligonucleotides are isolated by sequential treatment with thiophenol and ammonium hydroxide, purification by reverse-phase chromatography, and treatment with 80% acetic acid. The method is extremely fast (less than 2.5 h are needed for each nucleotide addition cycle), yields in excess of 95% per condensation are obtained, and isolation of the final product is a simple one-step column purification. The syntheses of d(C-G-T-C-A-C-A-A-T-T) and d(A-C-G-C-T-C-A-C-A-A-T) were carried out as a test of this method. Yields of support-bound deoxyoligonucleotides were 64% and 55%, the isolated yield of deoxydodecanucleotide was 30%. Both synthetic products were homogeneous and biologically active by every criteria so far tested.

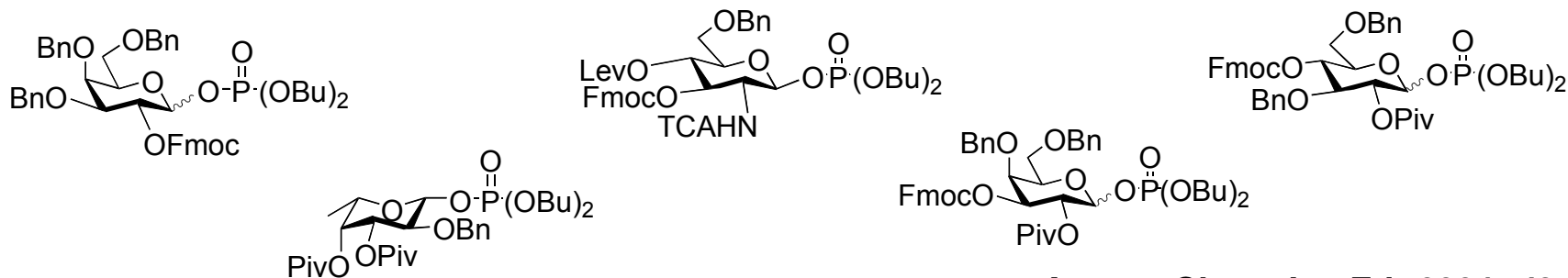
The Automated Oligosaccharide Synthesizer



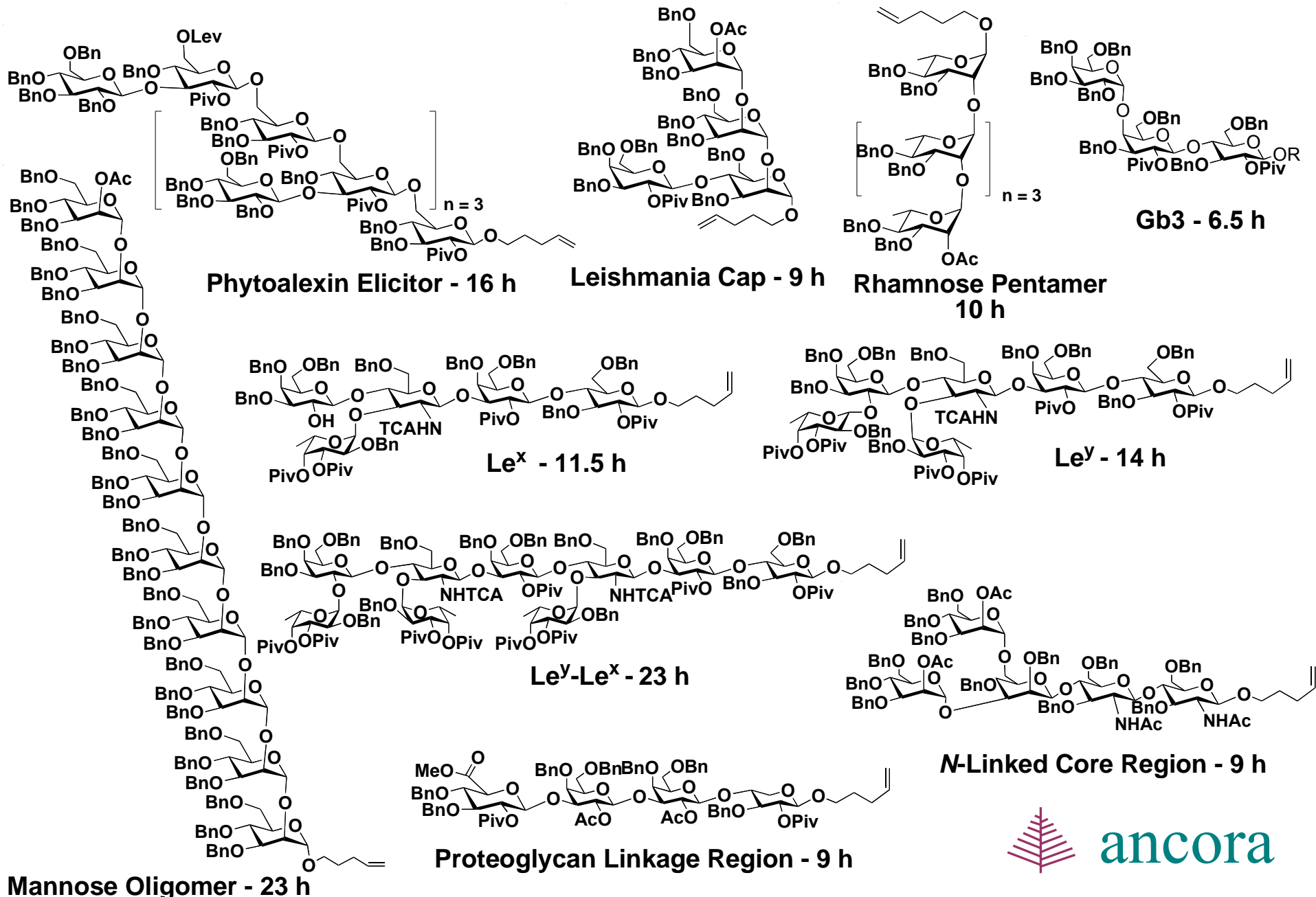
Blood Group Determinants and Tumor Associated Antigens



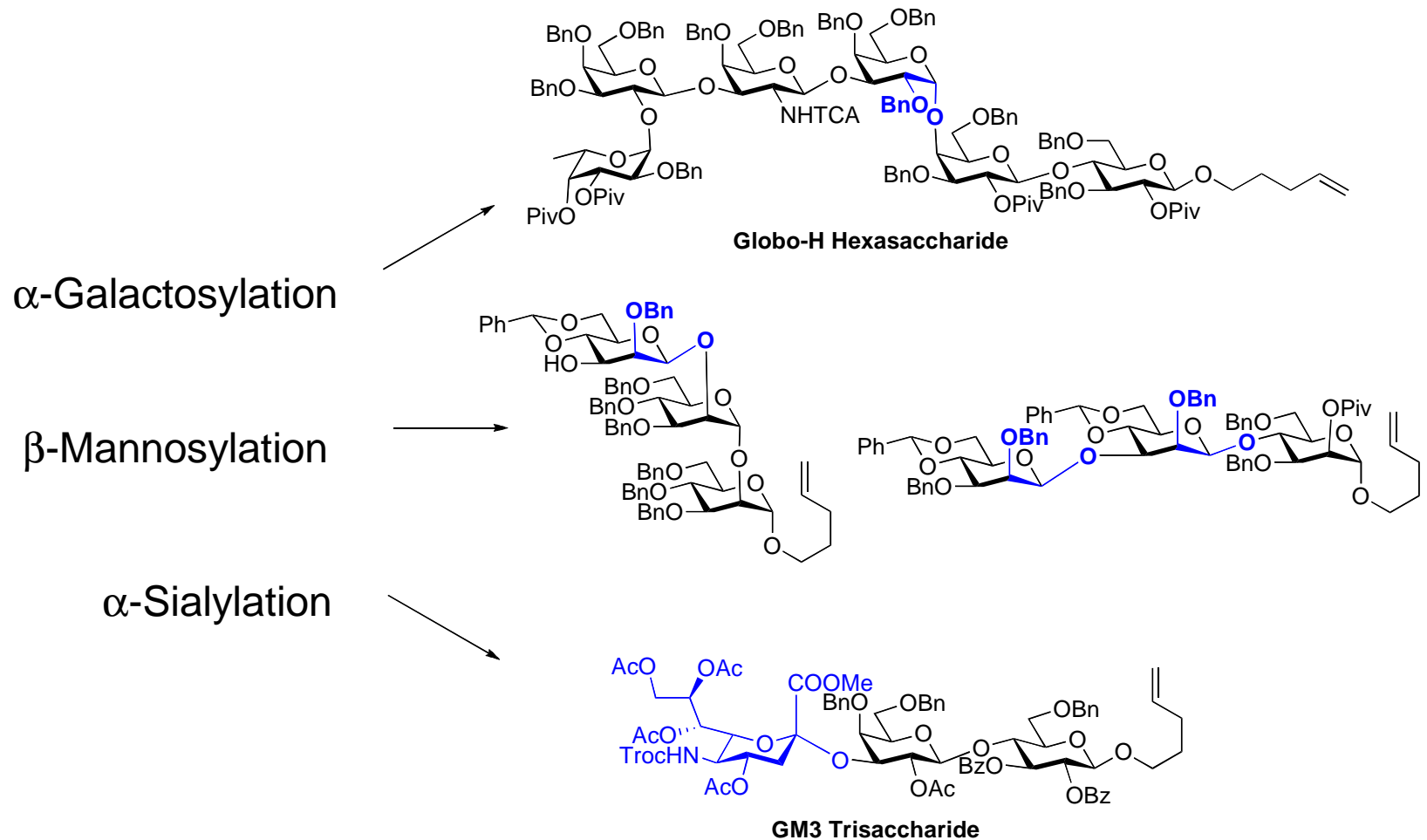
Le^yLe^x



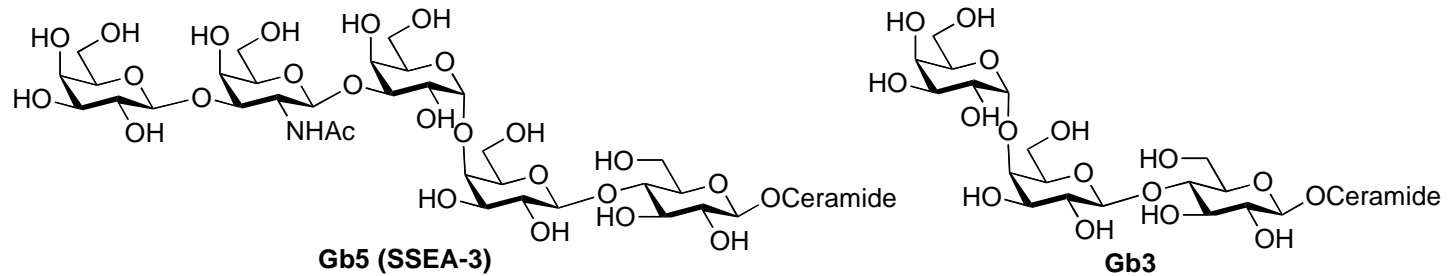
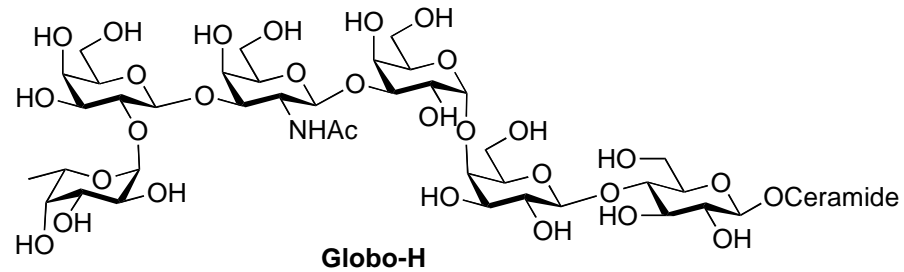
Automated Synthesis of Complex Structures



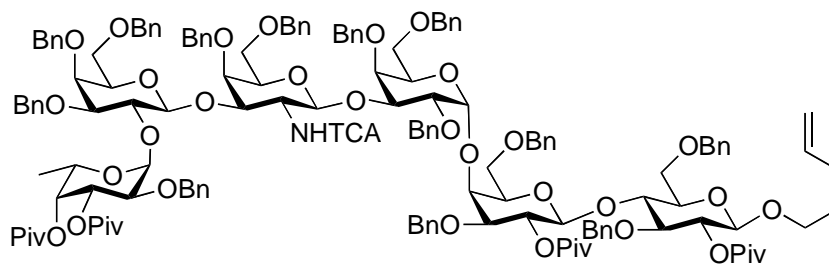
Automation of Difficult Glycosylations



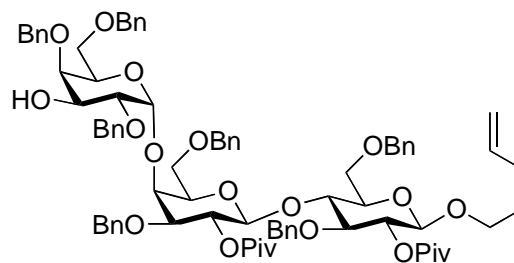
Globo-H Series of Tumor-Associated Antigens



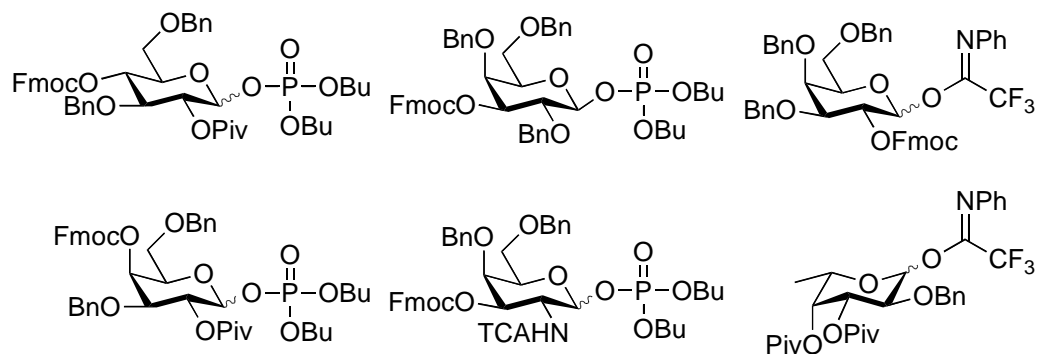
Globo-H: Retrosynthesis



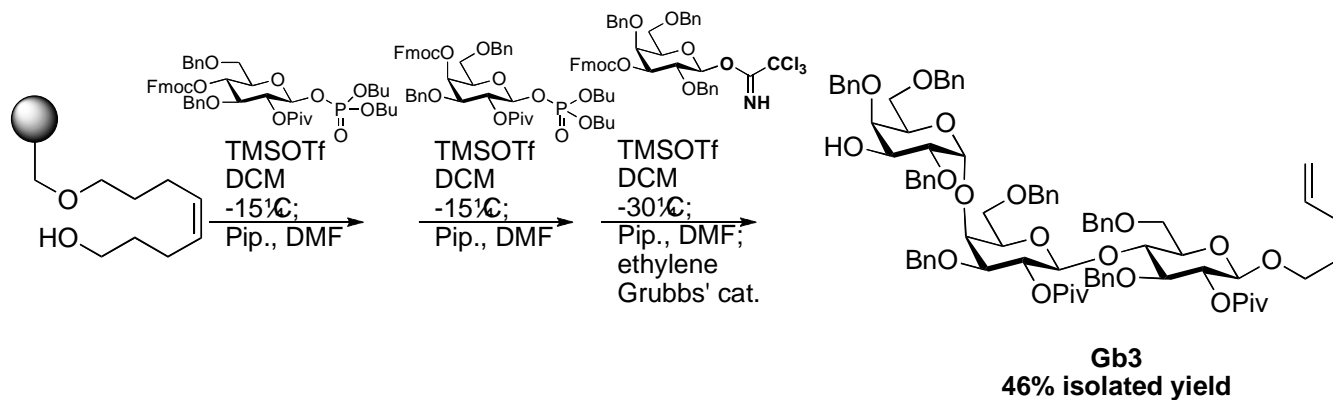
Globo-H Hexasaccharide



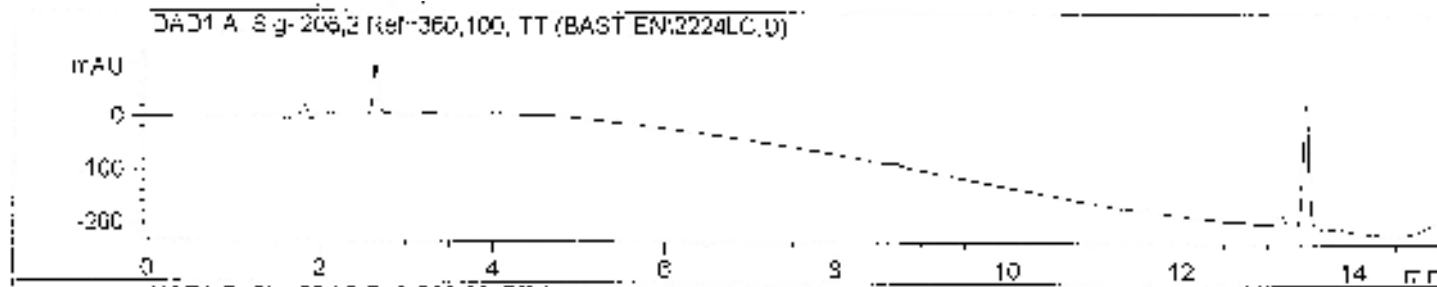
Gb3 Trisaccharide



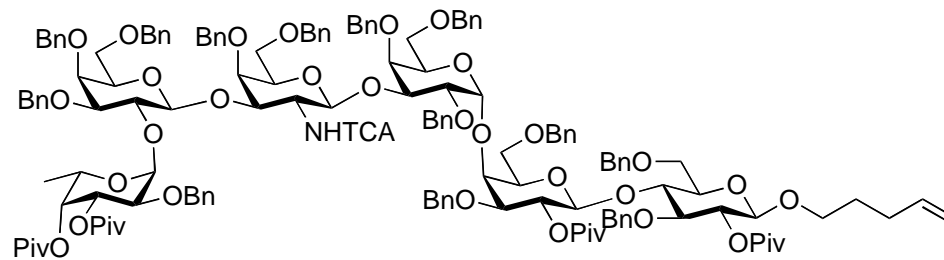
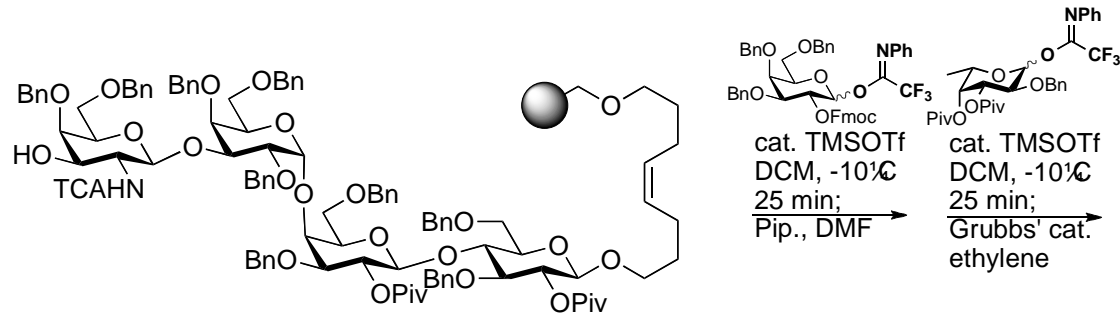
Gb3 synthesis



Crude HPLC

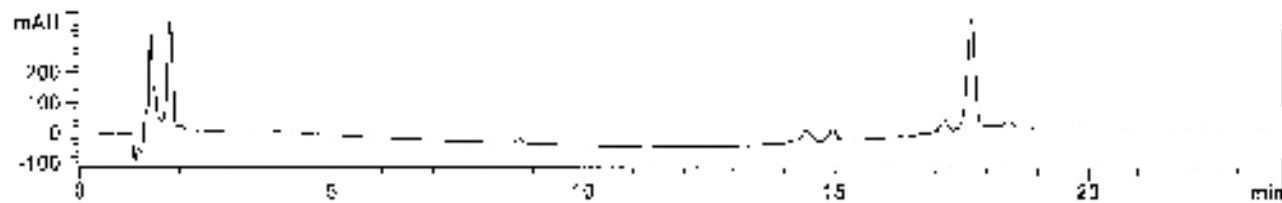


Globo-H Synthesis

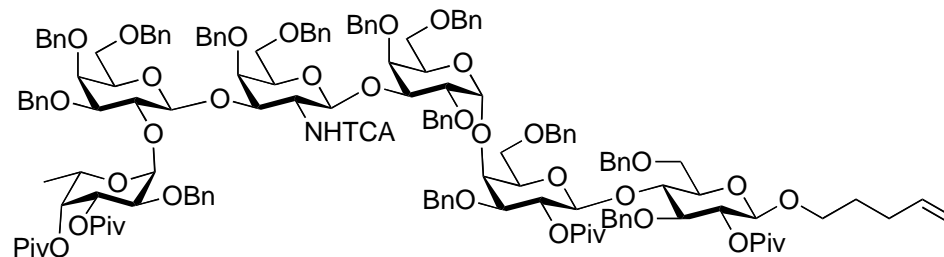


Globo-H Hexasaccharide

Crude HPLC

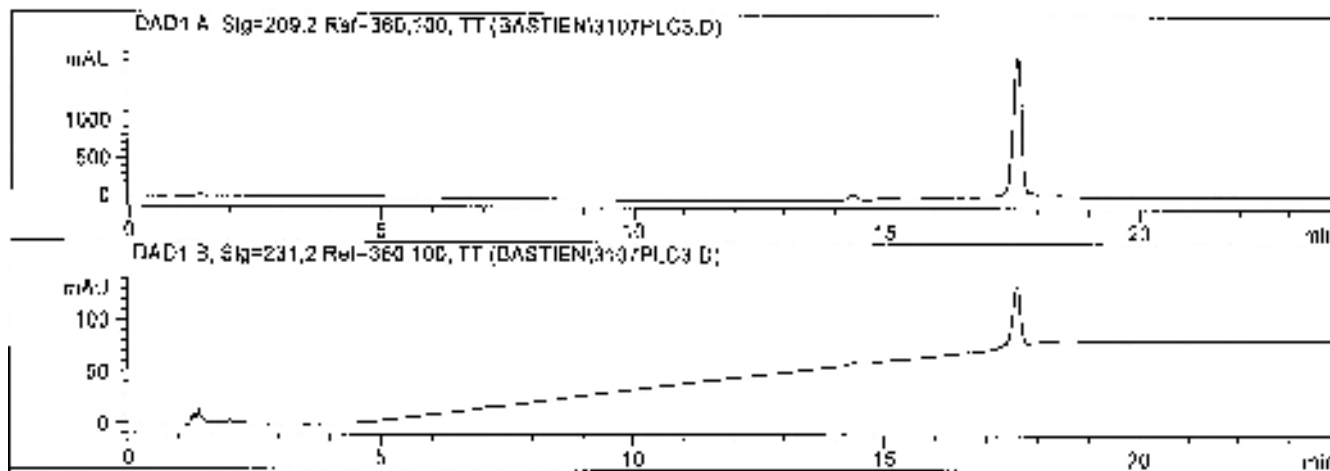


Purified Globo-H

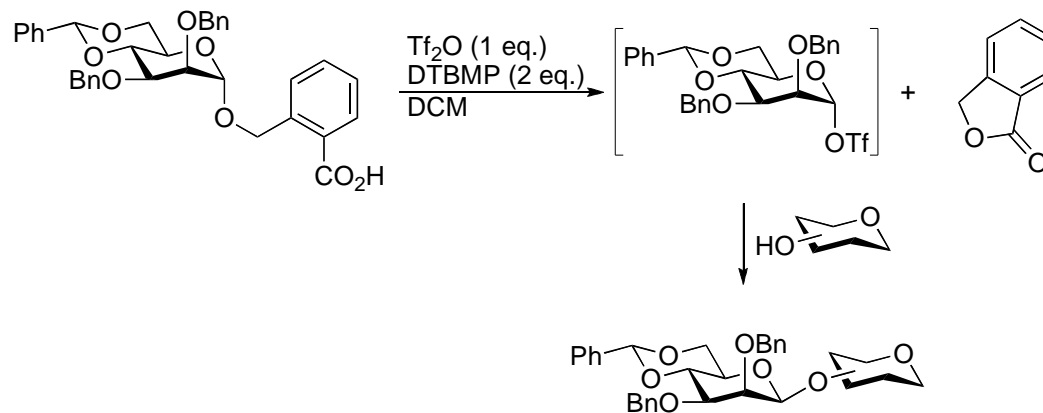


Globo-H Hexasaccharide

30% overall yield after column chromatography

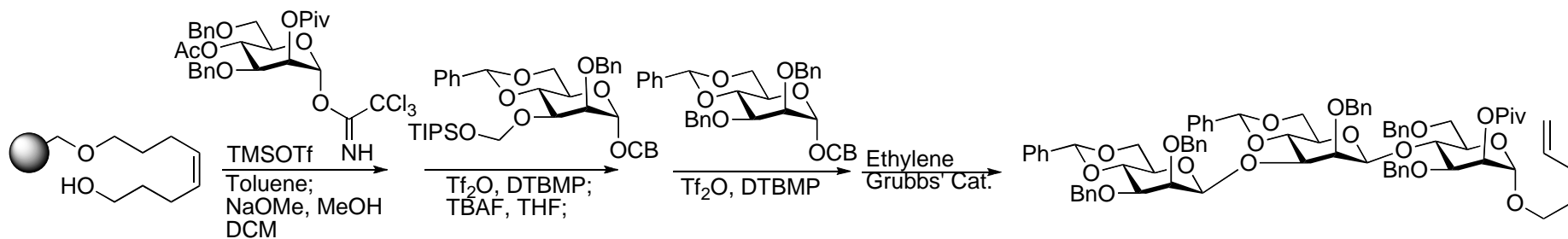


β -Mannosylation

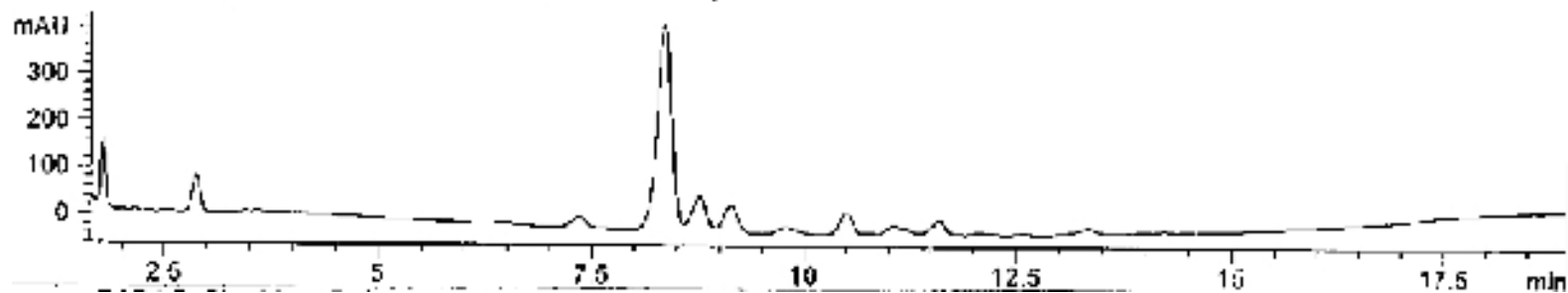


- No pre-activation necessary
- Compatible with linker olefin
- No acceptor by-product formation

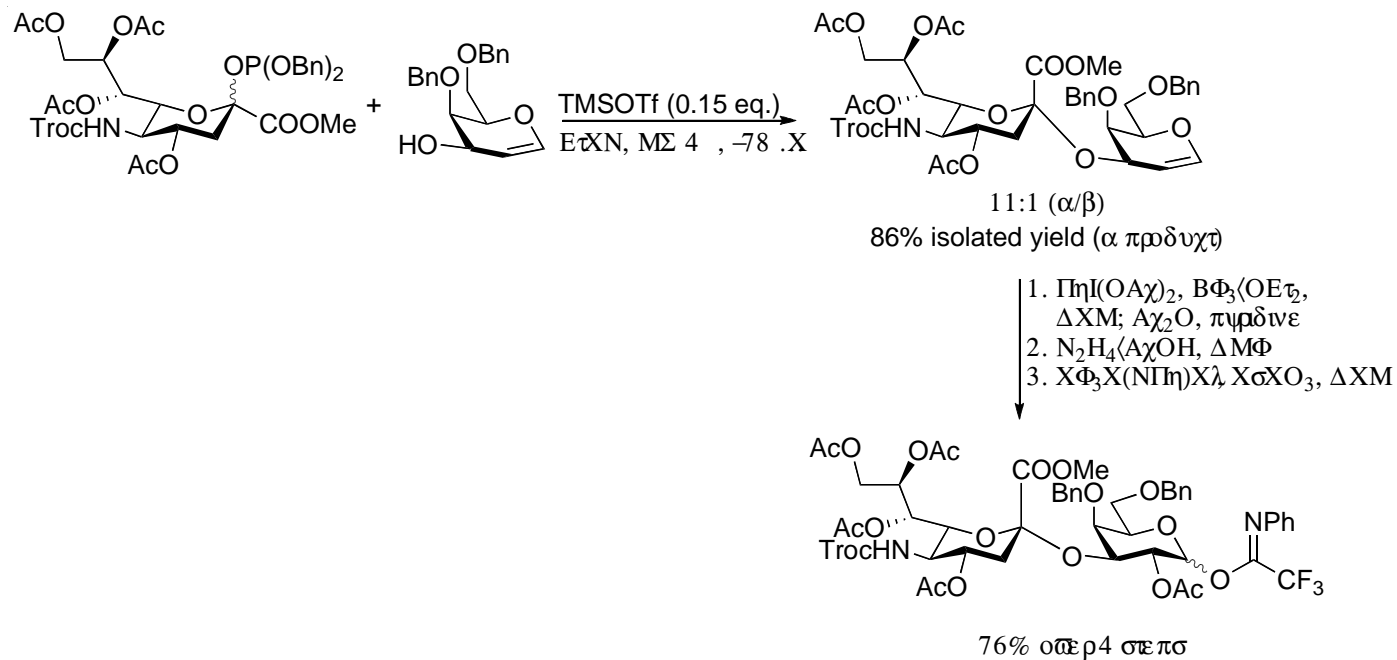
Elongation of the C3 Position



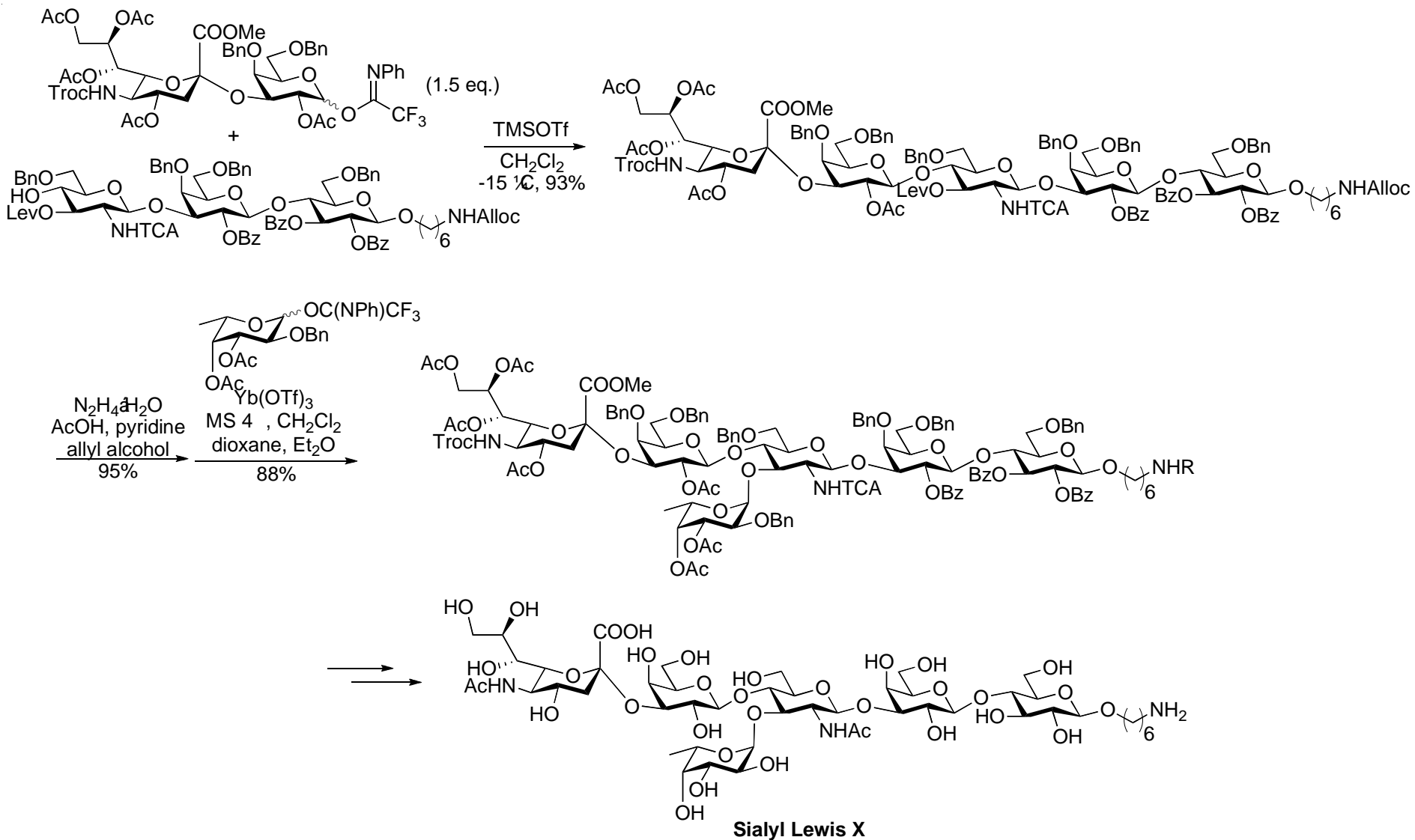
60% isolated yield (over 6 steps)



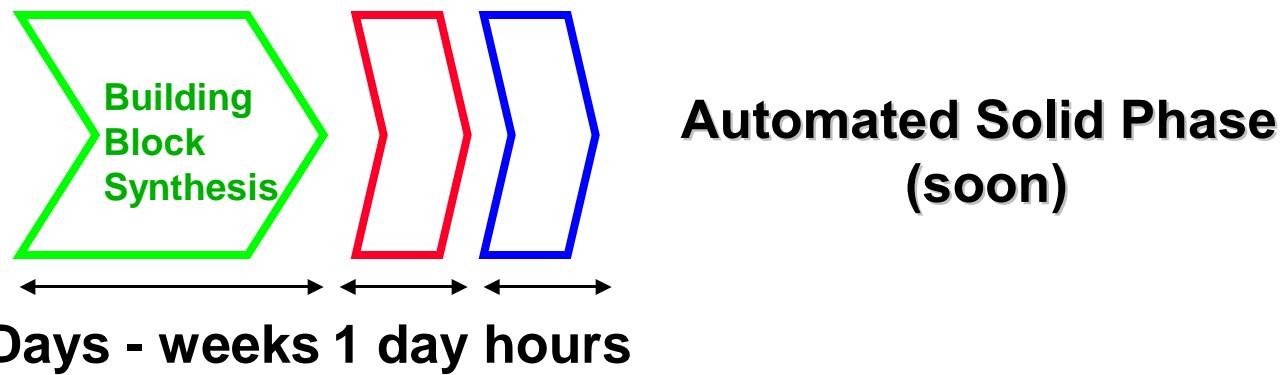
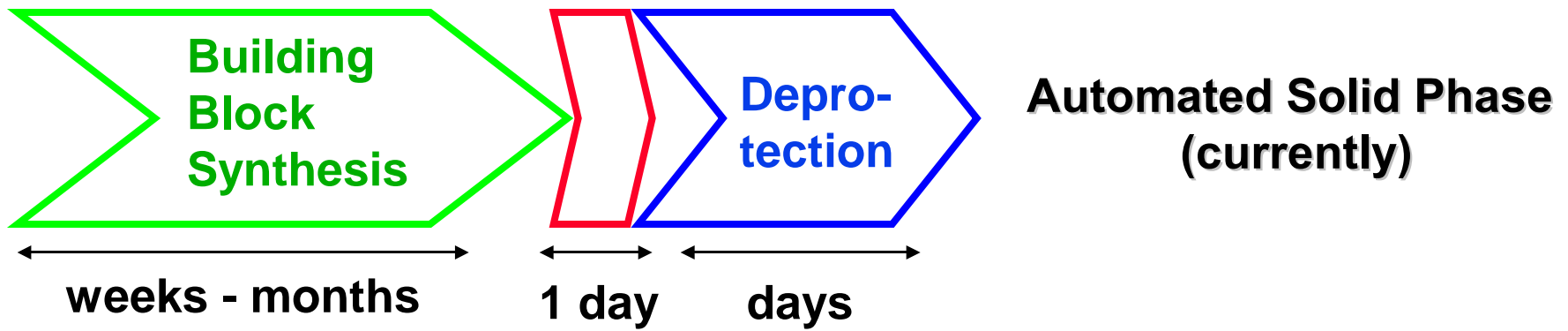
Sialic Acid Disaccharide Building Block



Solution Phase Sialyl Lewis X Synthesis



Time Allocation During Oligosaccharide Synthesis

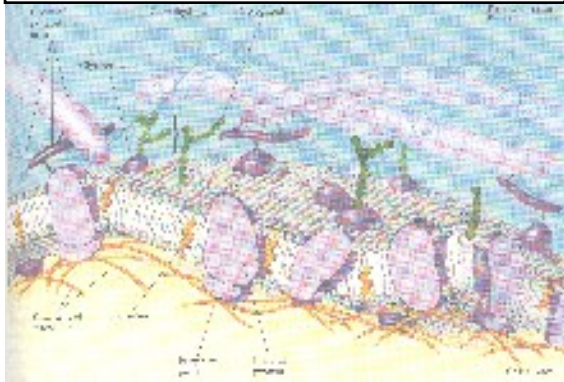


Synthetic Carbohydrate Antigens

**Development of Vaccine Candidates Against
Parasites, Bacteria and Cancer**

Carbohydrate Vaccine Development Path

Identify unique antigen



Synthesize antigen



Conjugate and formulate

M. tuberculosis

Avian flu
Bacterial antigens

Test Immunogenicity

Cancer antigens

Challenge animal model

Leishmania, HIV

B. anthracis

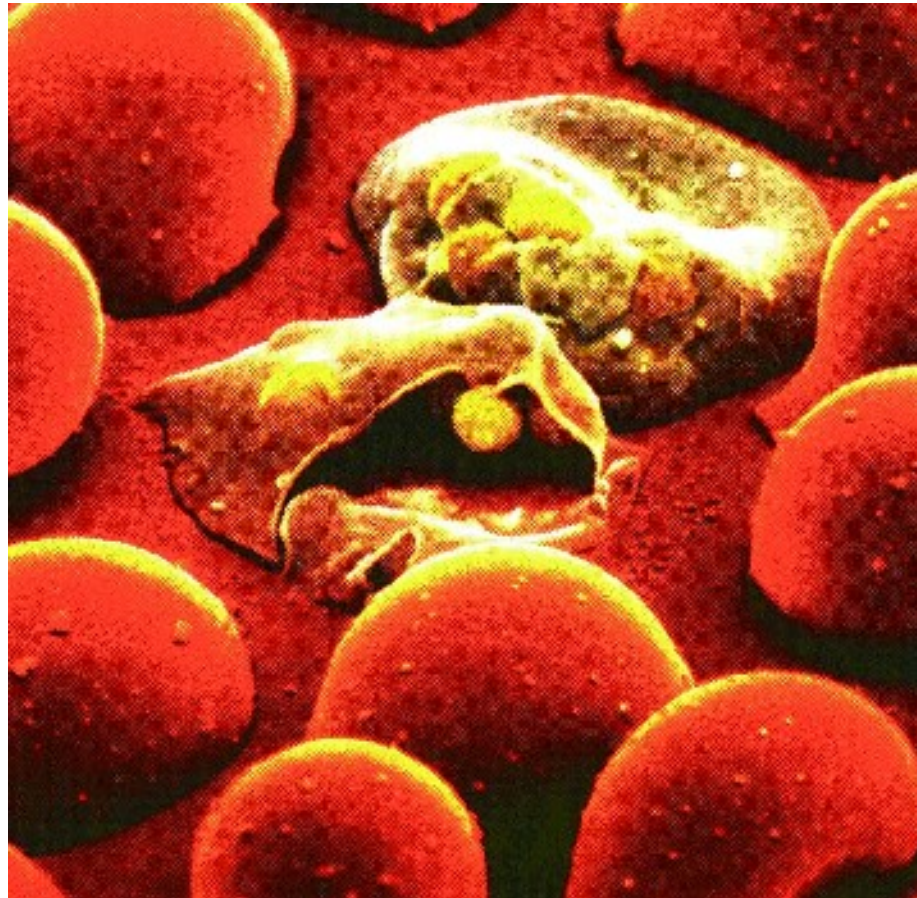
Preclinical Development

Malaria

Clinical Development



An Anti-Toxin Malaria Vaccine



Clinical and Anti-parasite Immunity to Malaria

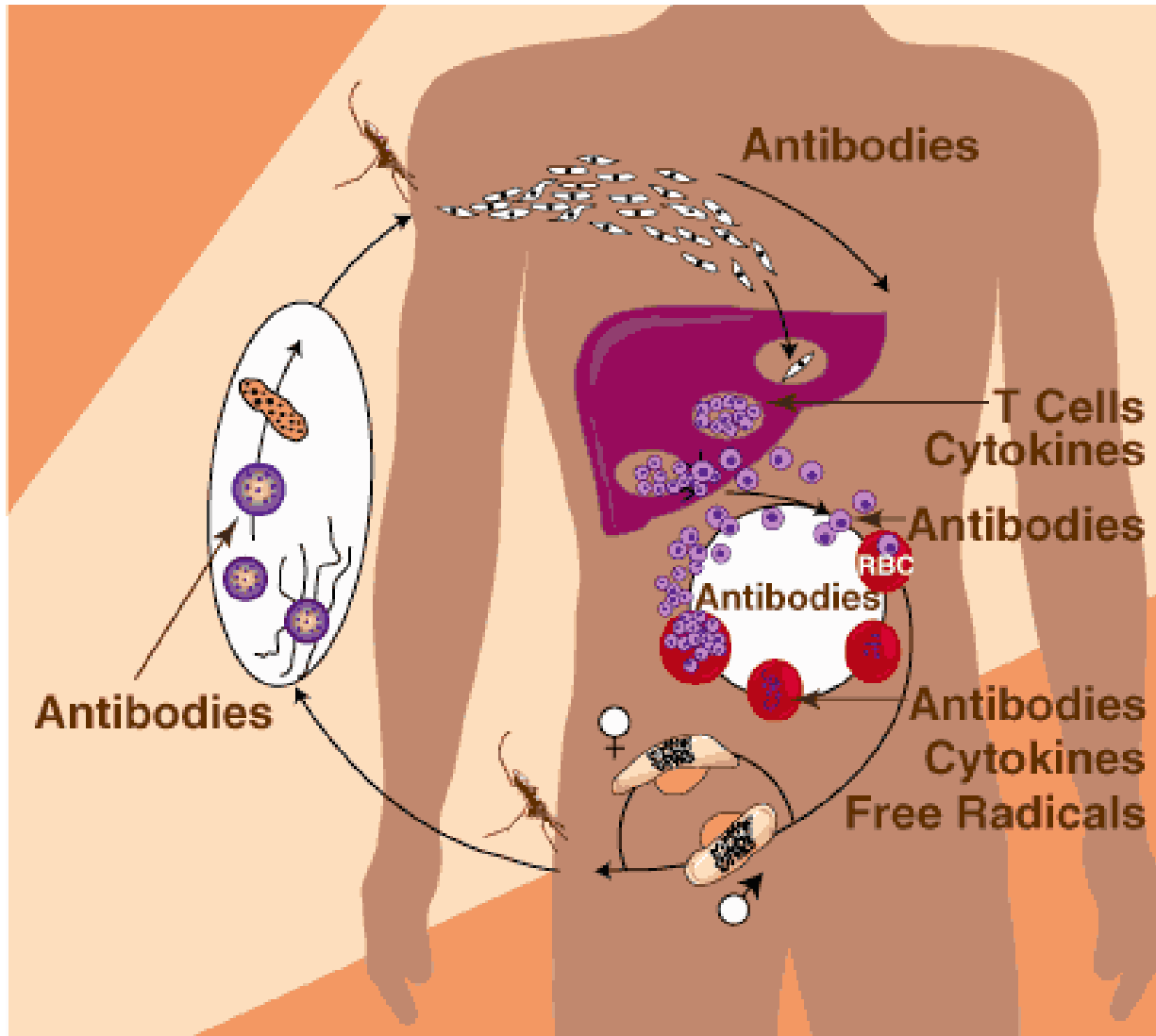
- many protein-based vaccines explored
- carbohydrate-based vaccines very successful against other diseases

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ARE NEEDED TO SEE THIS PICTURE.

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))

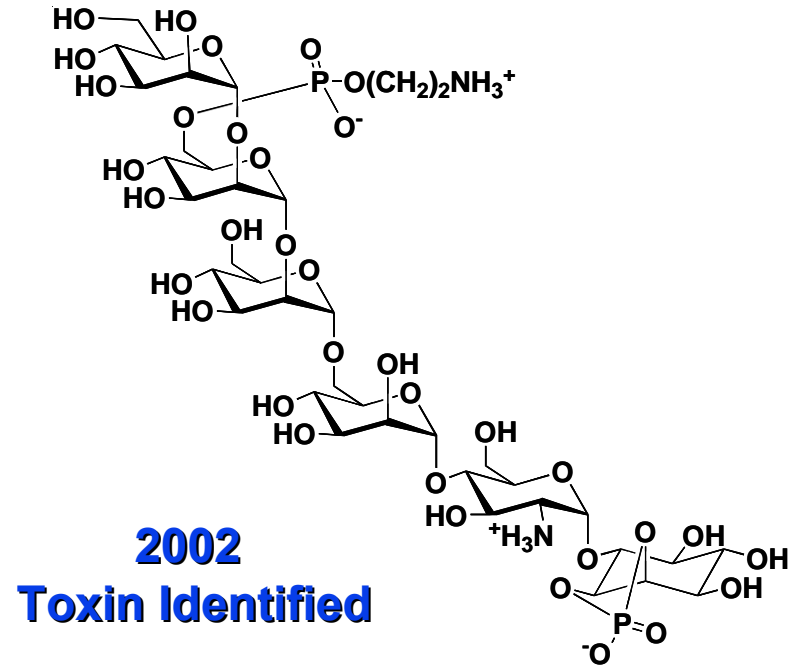
The *Plasmodium falciparum* Life Cycle



An Anti-Toxin Malaria Vaccine

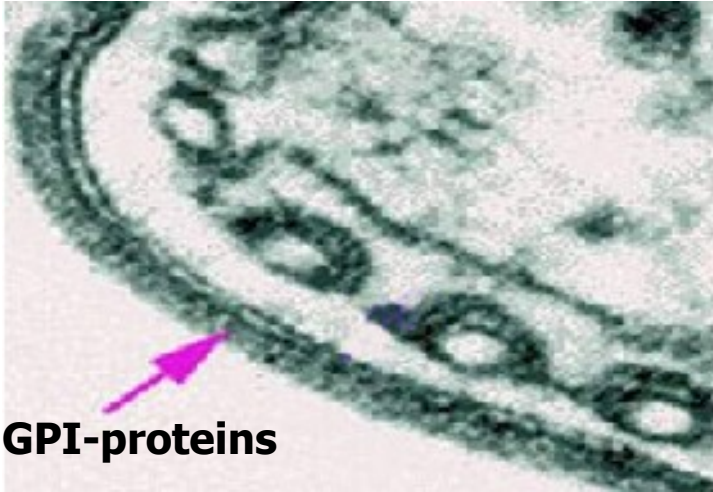
1896

Golgi Postulates Malaria Toxin



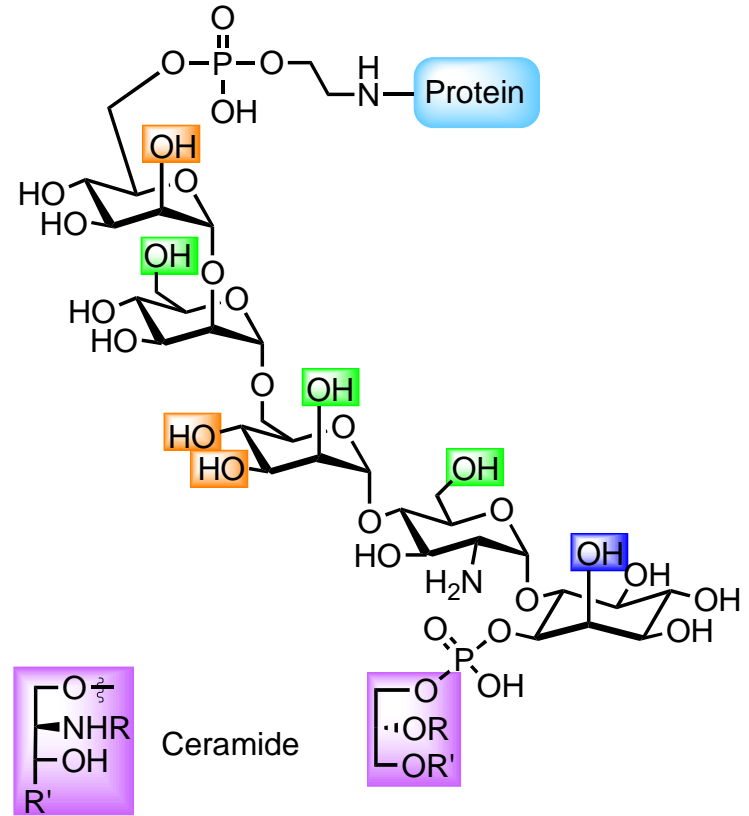
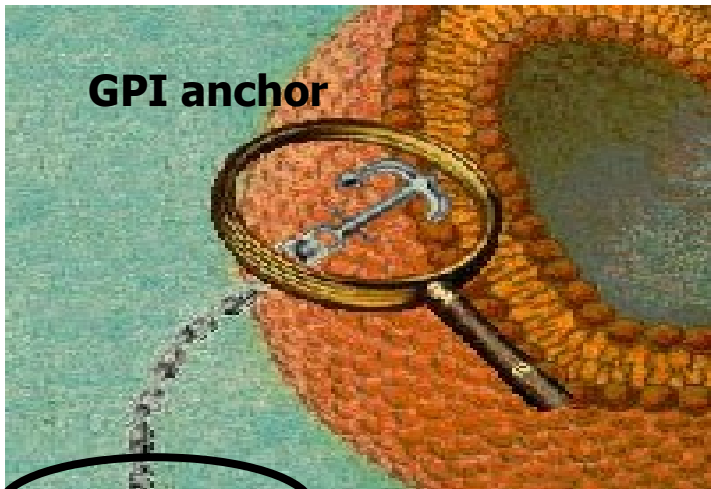
- 1) Substance isolated from *P. falciparum* - structure postulated
- 2) Synthesis of structure to confirm assignment
- 3) Use synthetic molecule as anti-toxin vaccine candidate

Glycosyl Phosphatidyl Inositol (GPI): Structure




> GPI-proteins

> Free GPIs



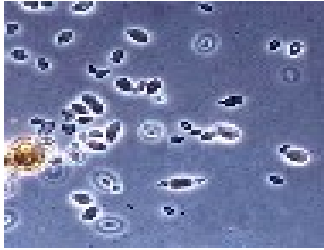
 Glycosylation

 EtN-Phosphorylation

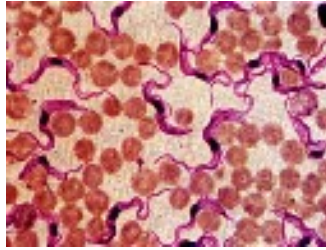
 Acylation

GPI-proteins

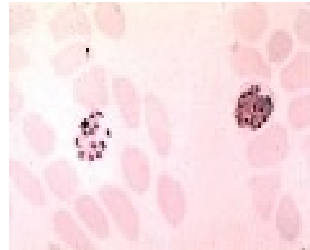
Glycosyl Phosphatidyl Inositol (GPI): Occurrence



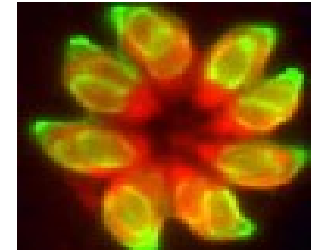
Yeast



T. brucei



P. falciparum



T. gondii



Human



High copy (10-20 Million per cell)



Low copy

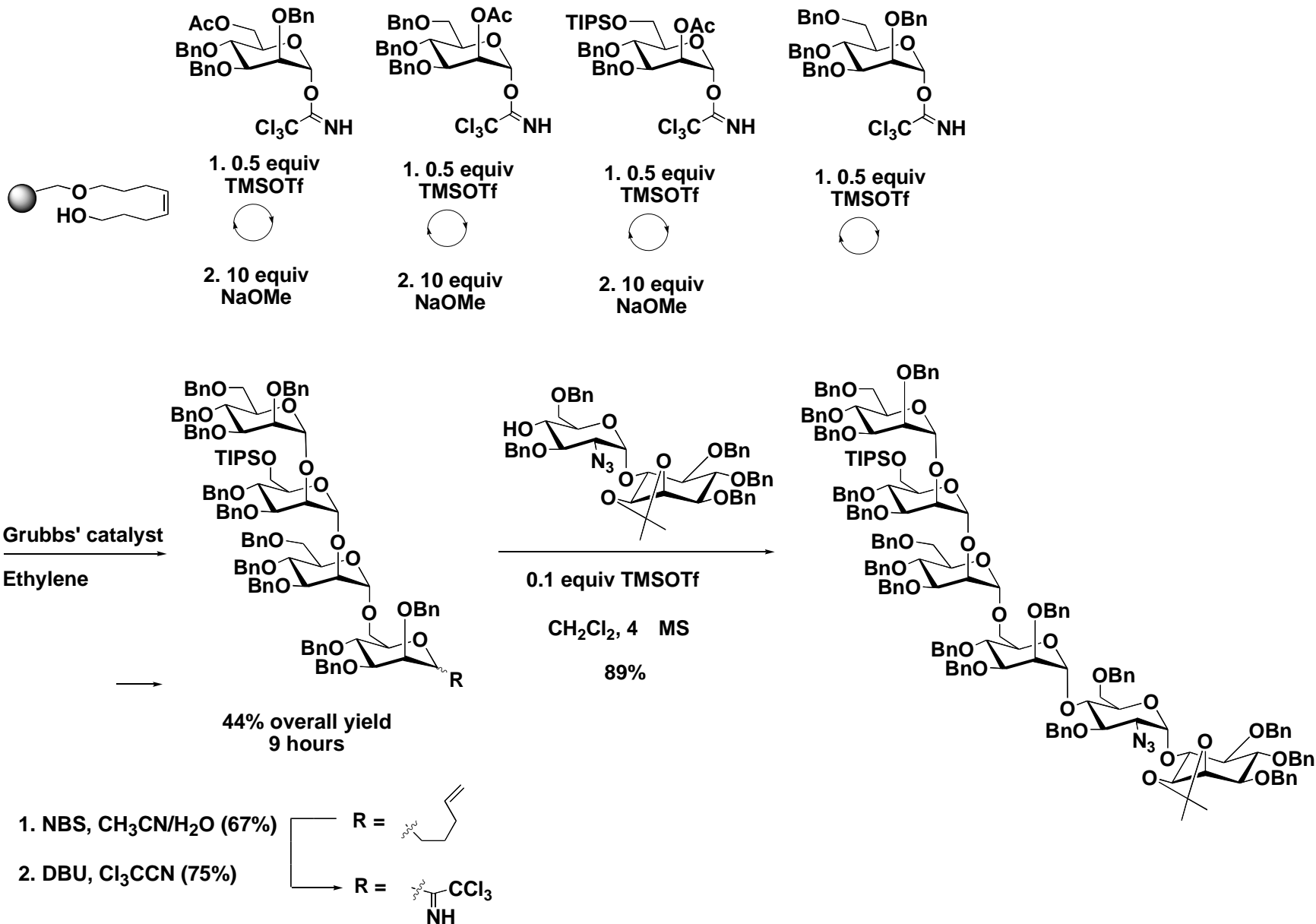
Exoenzymes e.g. alkaline phosphatase,

Adhesion molecules e.g. Neural cell adhesion molecules

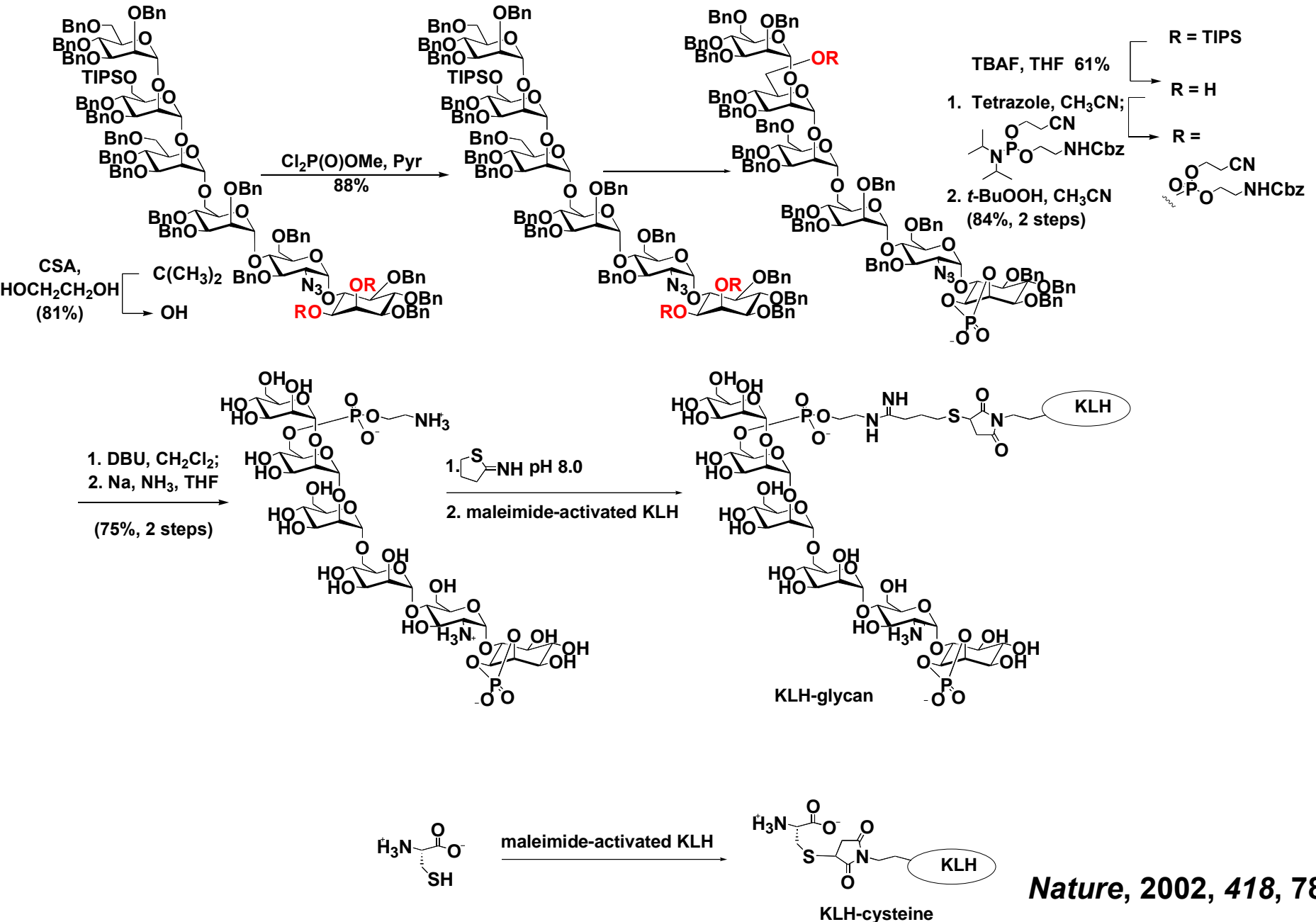
Complement regulatory proteins e.g. DAF, CD59

Protozoa surface antigens e.g. SAG1, MSP1

Semi-Automated Assembly of the GPI Glycan

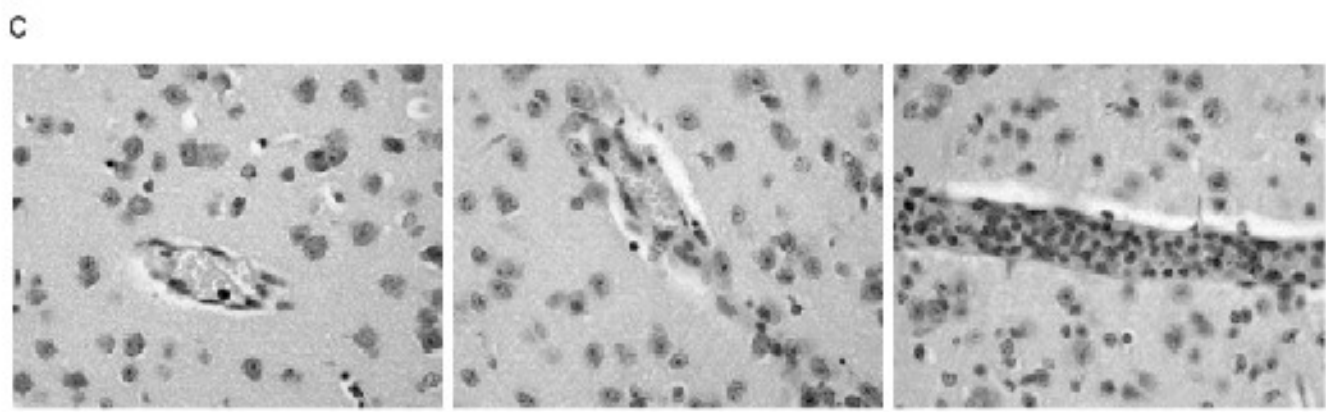
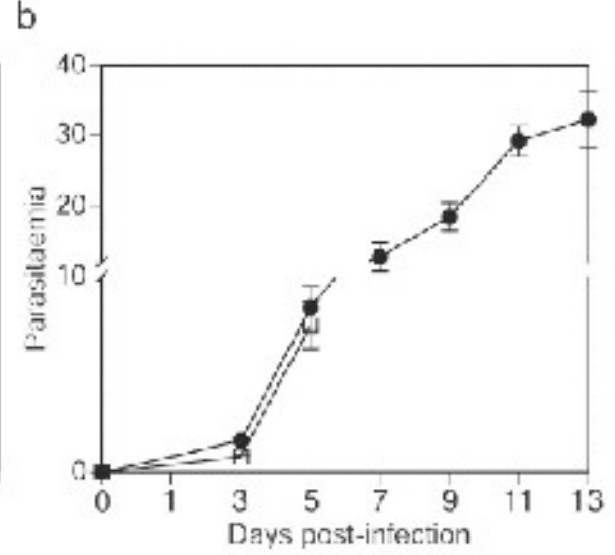
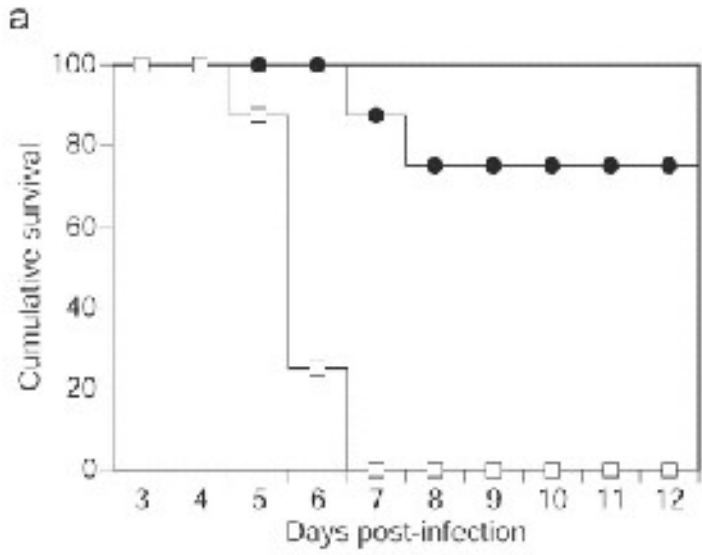


Synthesis of a Malaria Vaccine Candidate

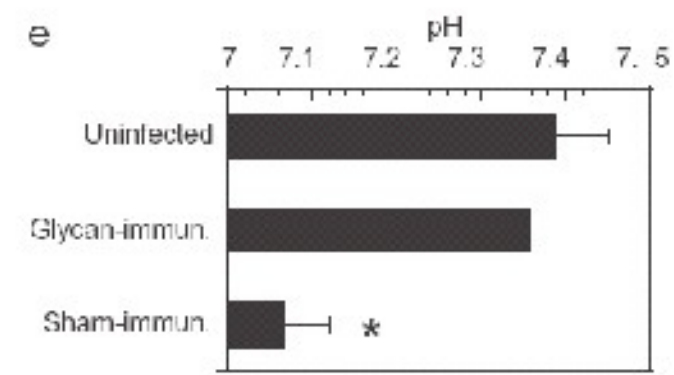
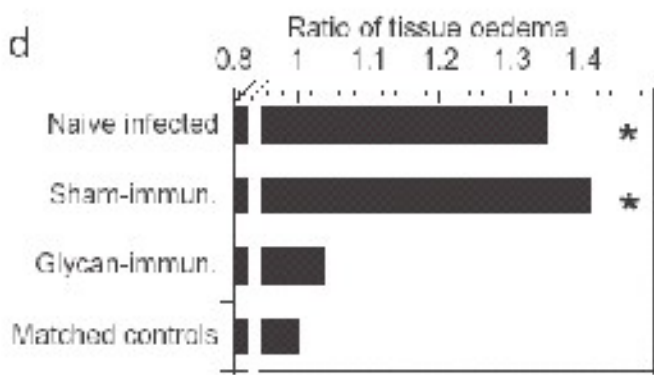


Vaccines vs Controls

Survival and parasitaemia



Cerebral Histology

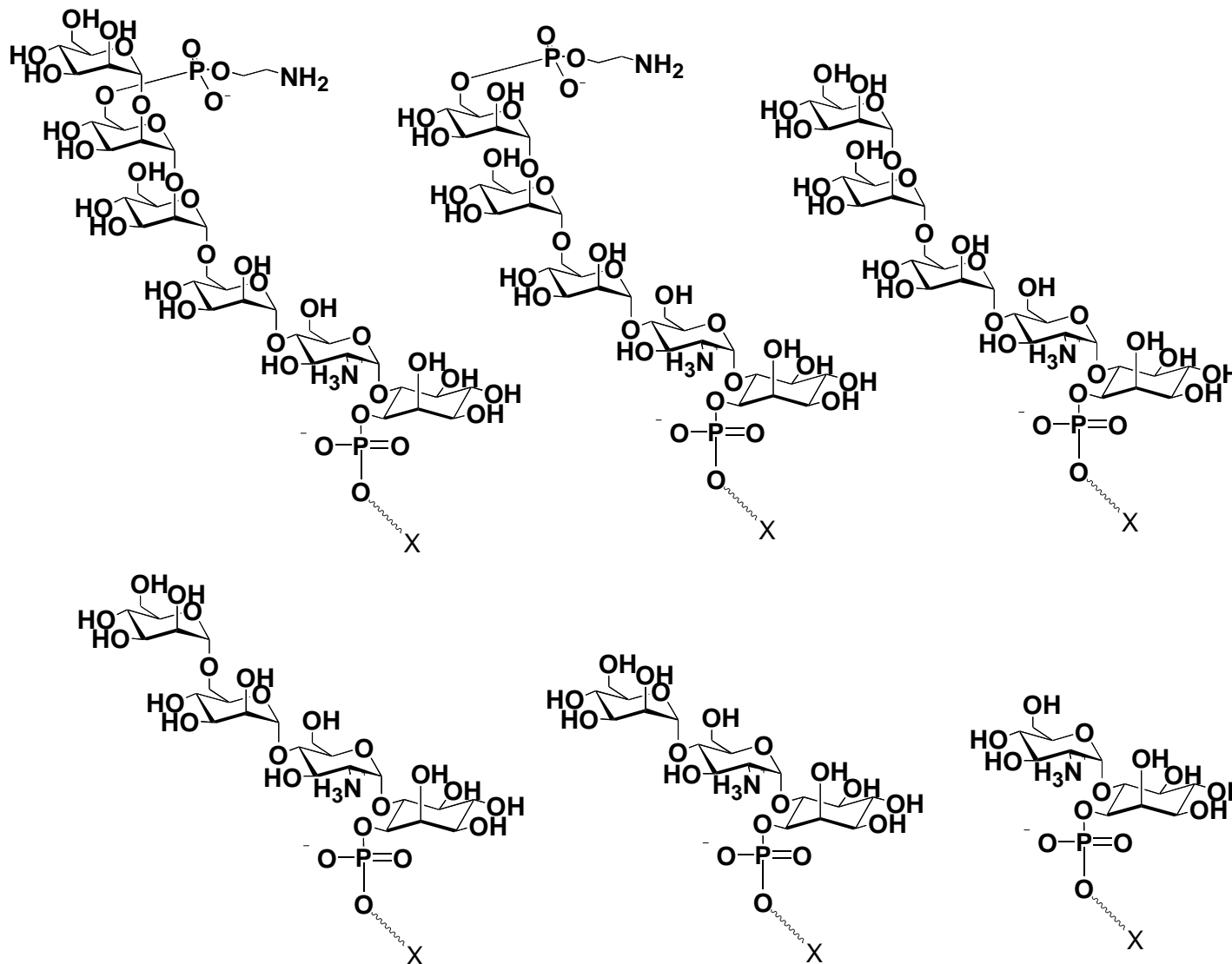


Systemic Pathology

Does an Anti-GPI Response Protect from Malaria Mortality?

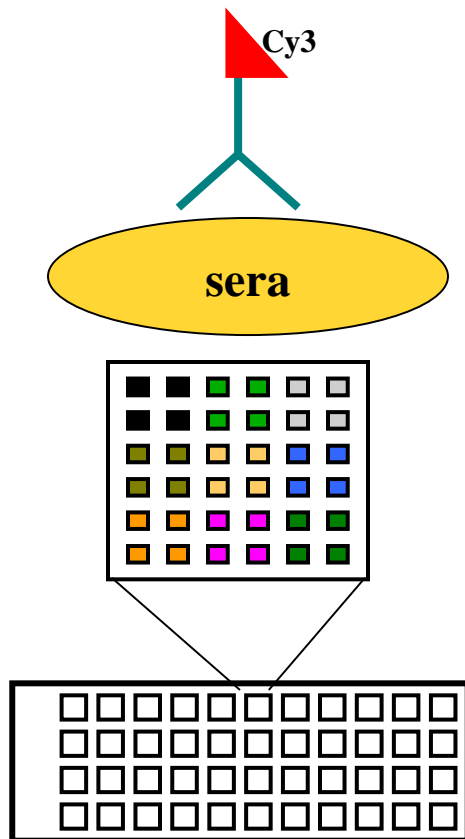
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Tools for Epitope Mapping & Biosynthesis Investigations

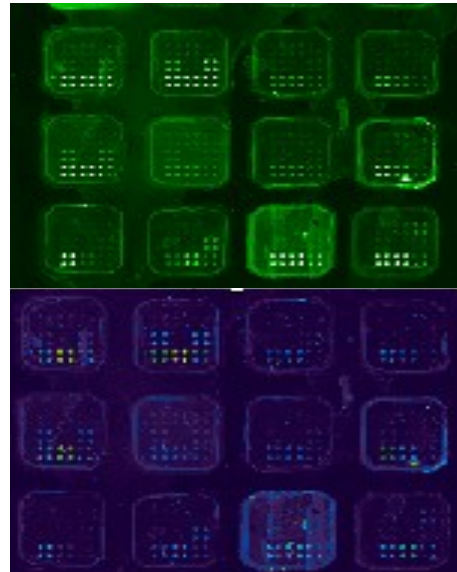


High Throughput Detection of Anti-GPI Antibodies on Microarrays

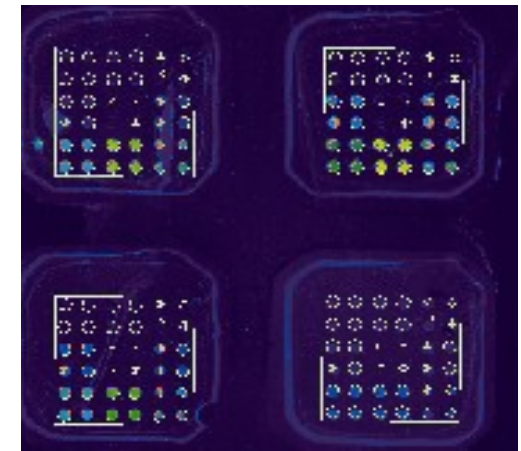
Incubation



Read Out

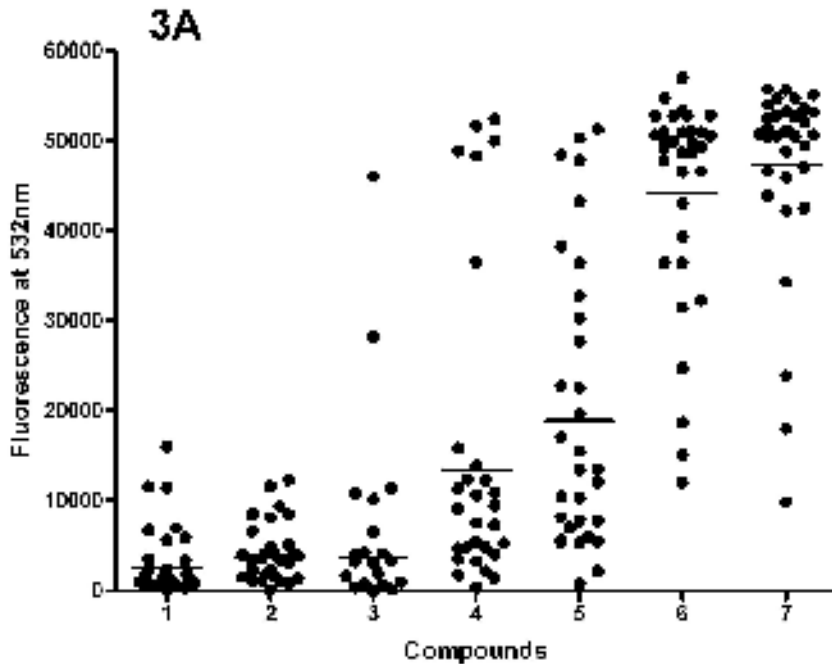


Quantification

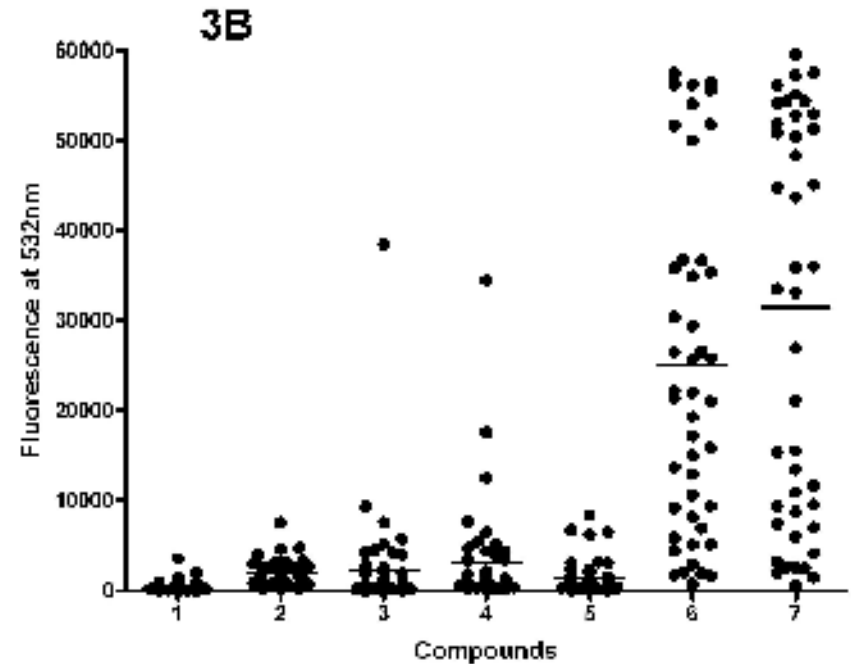


Mean Anti-GPI IgG Titres in Human Sera

Malaria endemic area of Burkina Faso



Malaria non-exposed Europeans



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
 - Scale-up and process development for synthetic antigen by **Ancora**

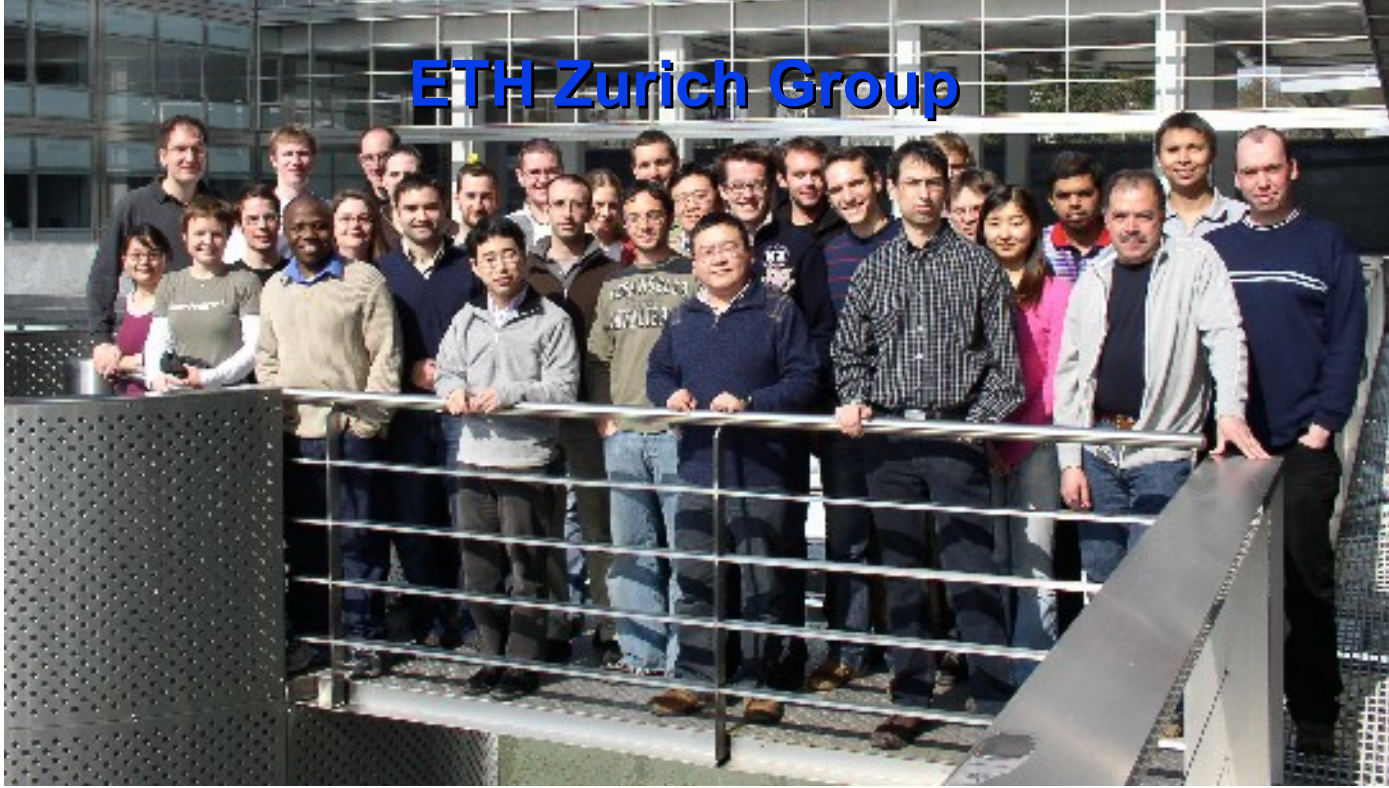
Synthesis	Total Yield	Linear Steps	Yield/Step	Scale
Seeberger Lab	0.26	26	79.5	10 -100 mg
Initial Ancora	2.70	27	87.5	1 - 100 g
Current Ancora	???	???	???	100 g - 5 kg

- 3) Conjugation and formulation agreement with major vaccine manufacturer
- 4) Toxicology and preclinical studies
- 5) Selection of sites for active and passive immunization trials

How do Merozoites Enter Red Blood Cells?

How Does *P. falciparum* Initiate the Inflammatory Response?

ETH Zurich Group



ETH

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Prof. Chatterjee
Prof. Schachner
Prof. Hengartner

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DFG (Emmy Noether Program)
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HFSP
EMBO
Roche Foundation
Fondation Bay

