Automated Oligosaccharide Synthesis

Peter H. Seeberger

ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

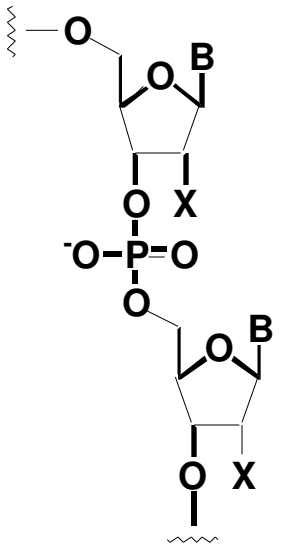
BURNHAM INSTITUTE
for MEDICAL RESEARCH
From Research, the Power to Cure

Biopolymers: Overview

Genomics

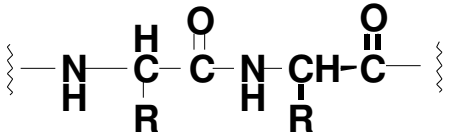
Proteomics

Glycomics

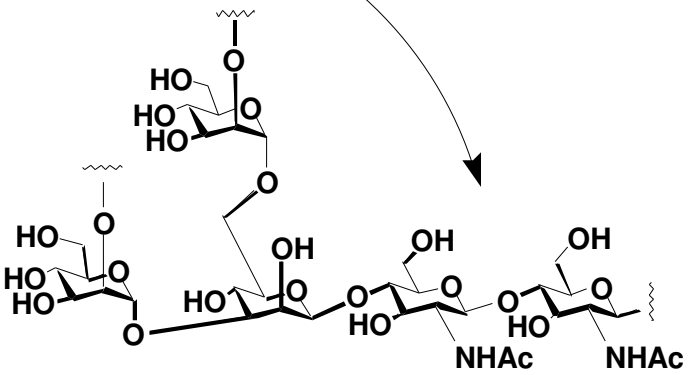


Transcription

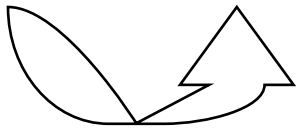
Translation



Glycosyltransferases



Replication

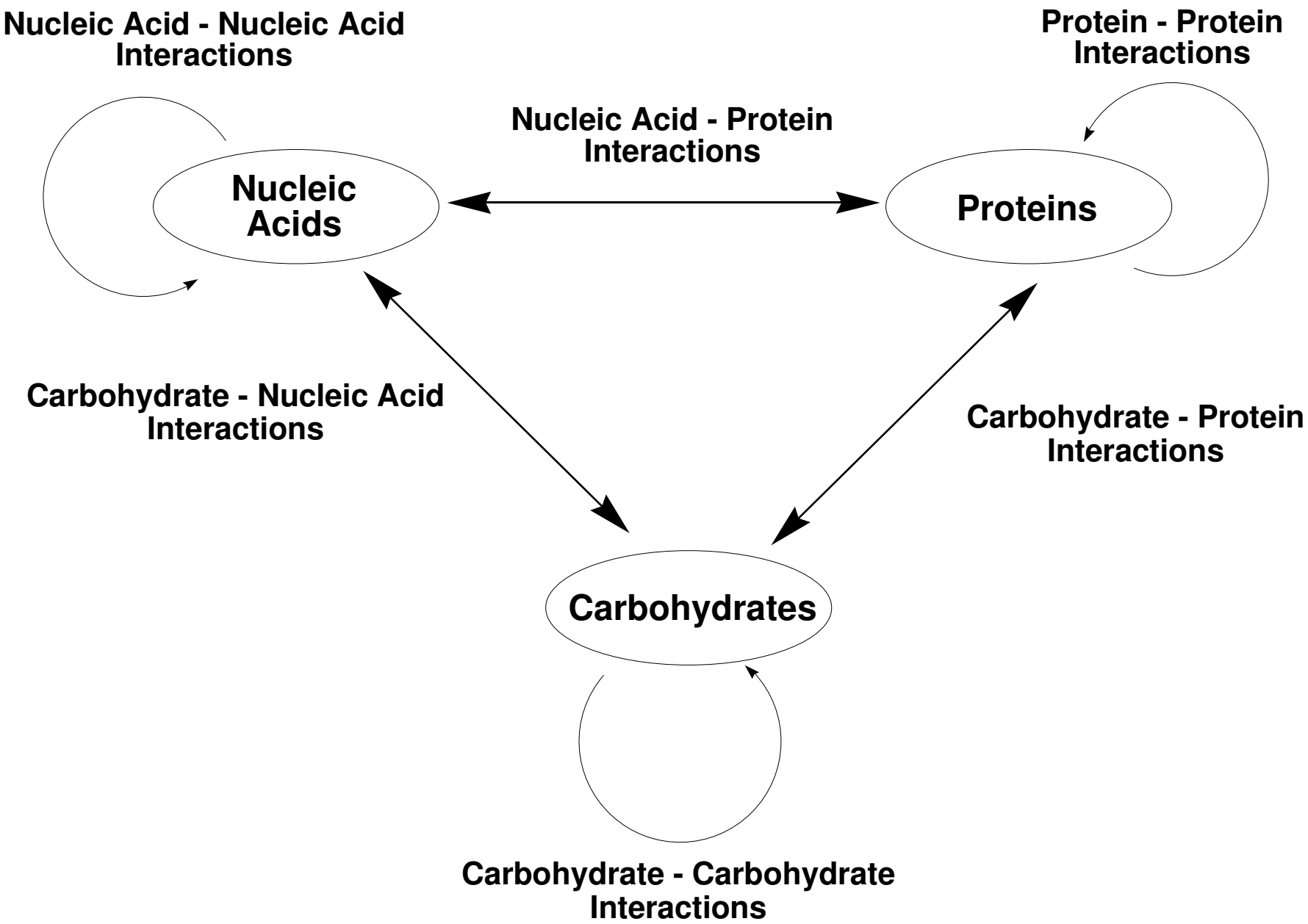


Nucleic Acids

Proteins

Oligosaccharides - Glycoconjugates

Biopolymer Interactions



J. Mol. Biol. (1972) **72**, 209–217

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[g], T. TERAO[h], H. WEBER[i] AND T. YAMADA[j]

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.

J. Am. Chem. Soc. **1981**, *103*, 3185–3191

Synthesis of Deoxyoligonucleotides on a Polymer Support[1]

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

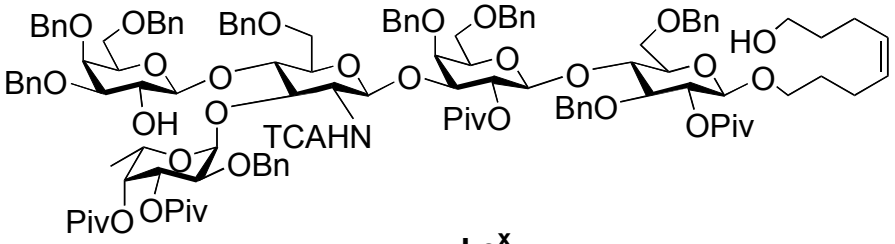
Contribution from the Department of Chemistry, University of Colorado, Boulder, Colorado 80309. *Received September 18, 1980*

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 T) were carried out as a test of this method. Yields of support-bound deoxyoligonucleotides were 64% and 55%, the isolated yield of deoxydocanucleotide was 30%. Both synthetic products were homogeneous and biologically active by every criteria so far tested.

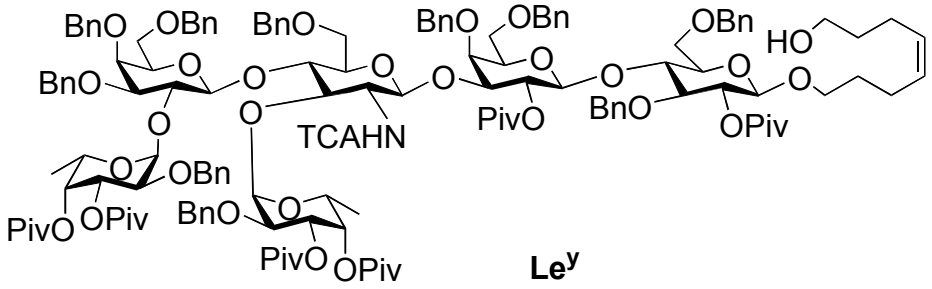
The Automated Oligosaccharide Synthesizer

Science 2001, *291*, 1523

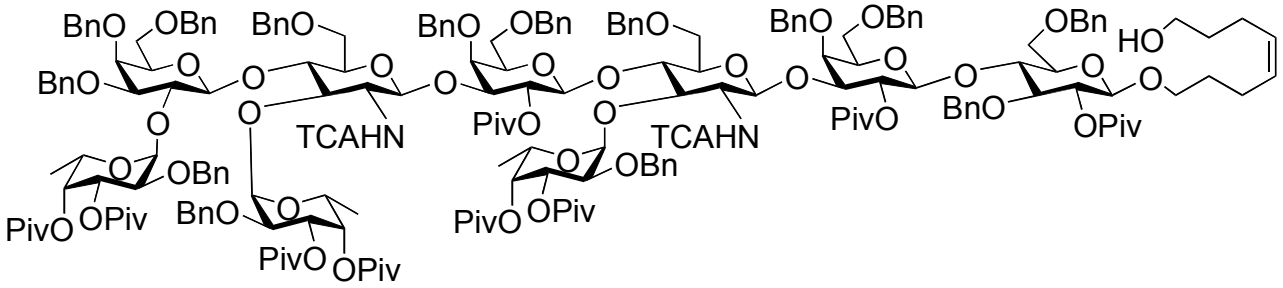
Blood Group Determinants and Tumor Associated Antigens



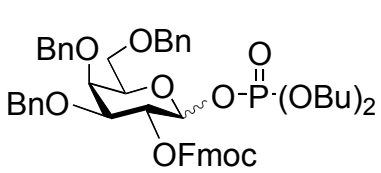
Le^x

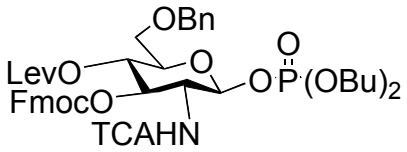


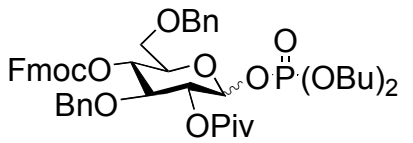
Le^y

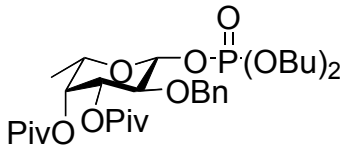


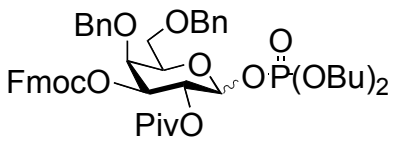
Le^{y}Le^x





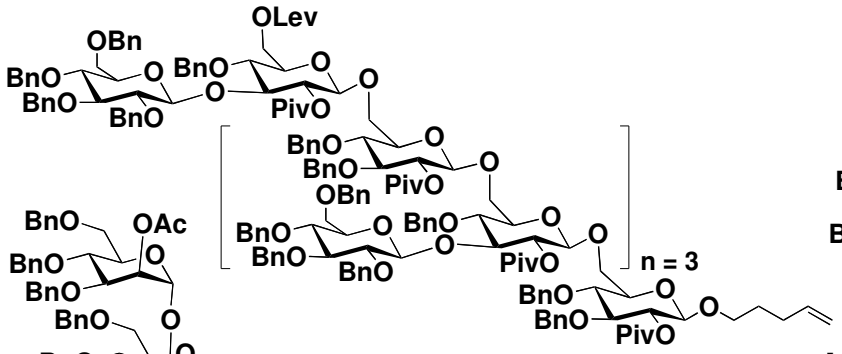




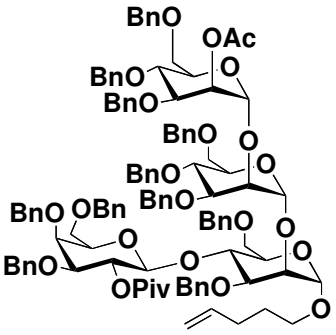


Angew. Chem. Int. Ed. **2004, *43*, 602**

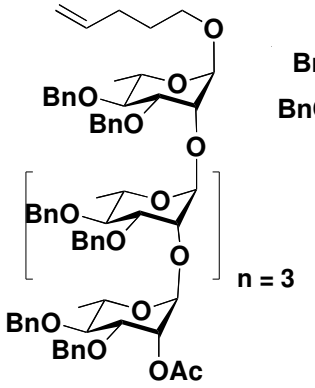
Automated Synthesis of Complex Structures



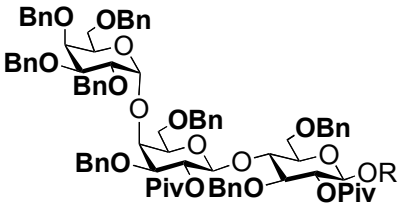
Phytoalexin Elicitor - 16 h



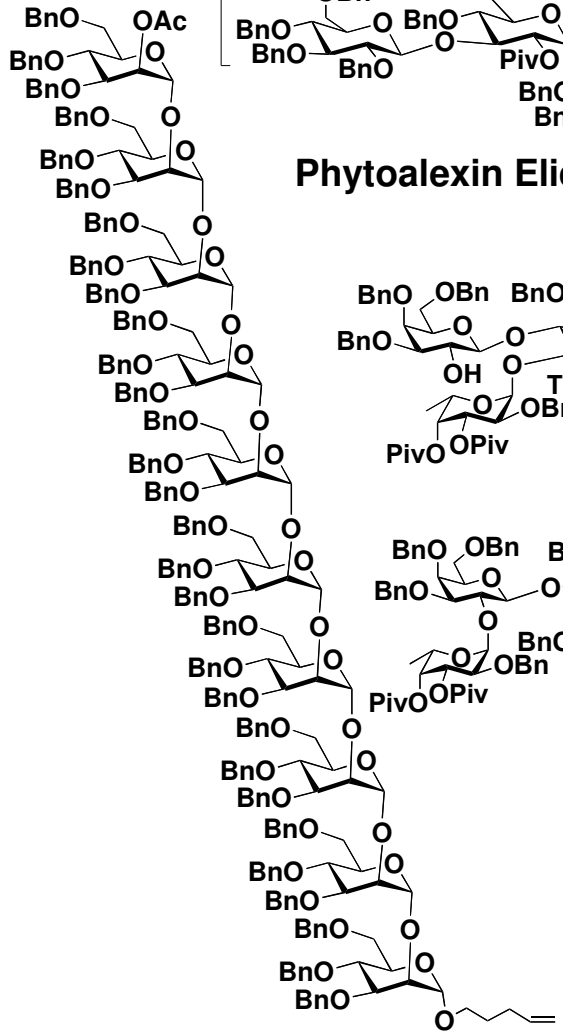
Leishmania Cap - 9 h



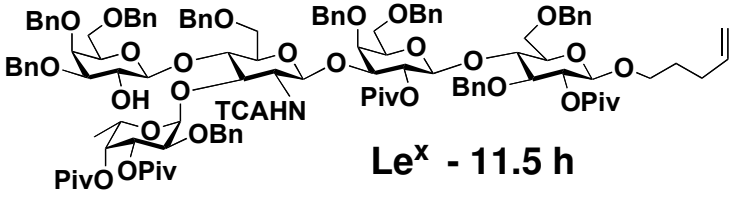
Rhamnose Pentamer
10 h



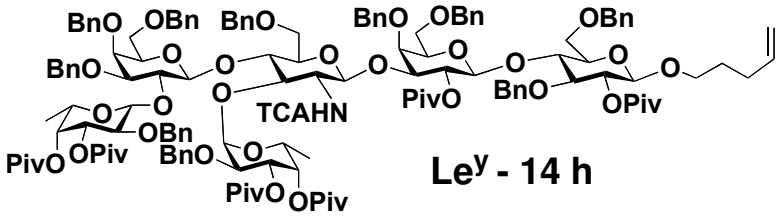
Gb3 - 6.5 h



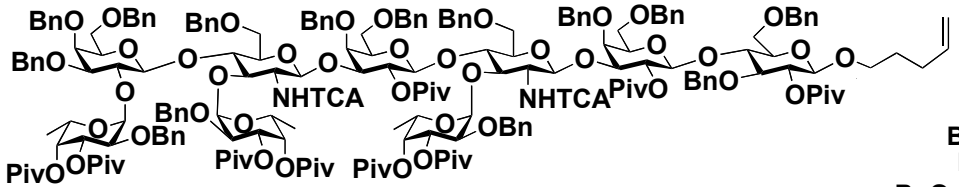
Mannose Oligomer - 23 h



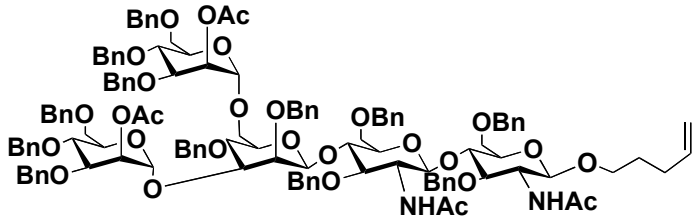
Le^{x} - 11.5 h



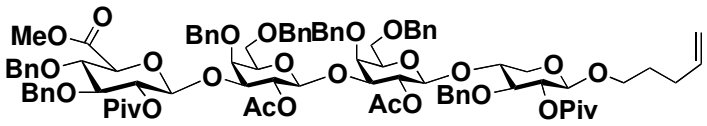
Le^{y} - 14 h



Le^{y}-Le^{x} - 23 h



N-Linked Core Region - 9 h



Proteoglycan Linkage Region - 9 h

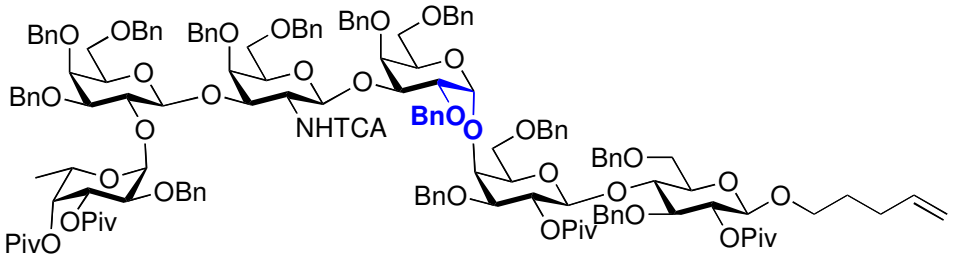
ancora

Automation of Difficult Glycosylations

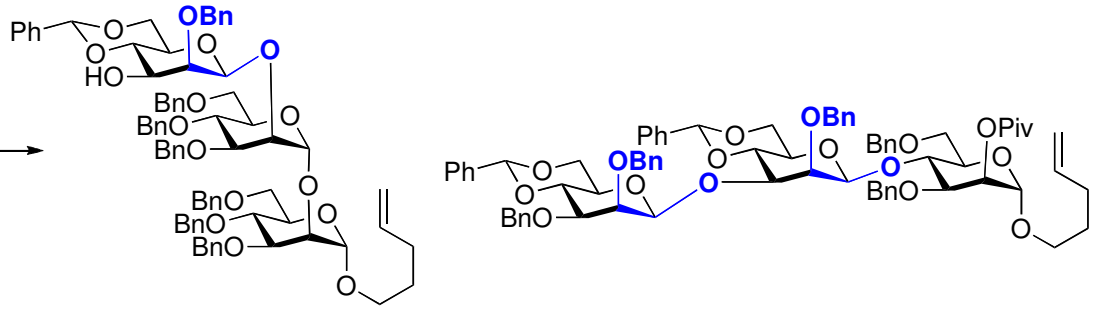
α-Galactosylation

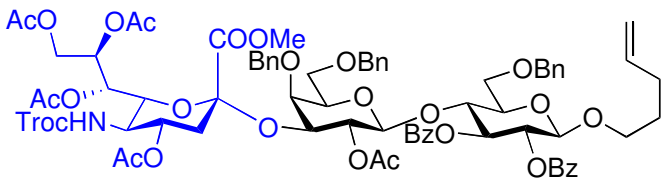
β-Mannosylation

α-Sialylation



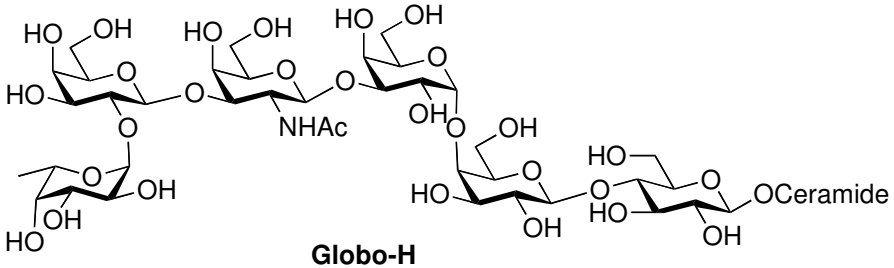
Globo-H Hexasaccharide



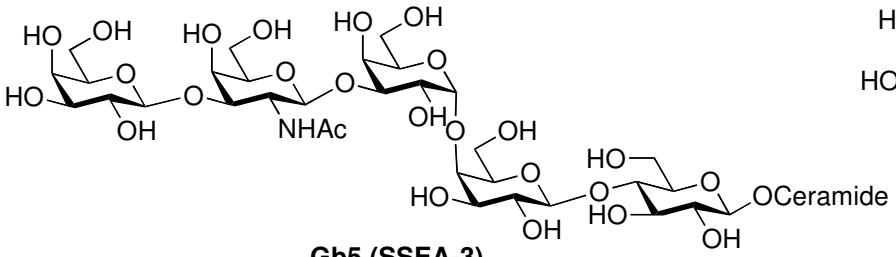


GM3 Trisaccharide

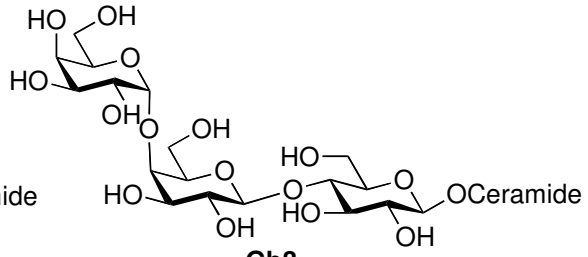
Globo-H Series of Tumor-Associated Antigens



Globo-H

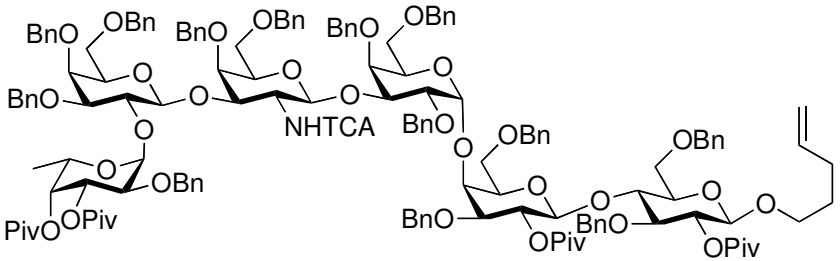


Gb5 (SSEA-3)

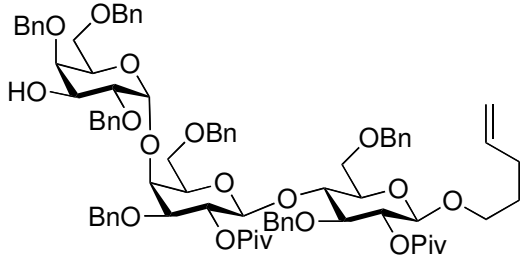


Gb3

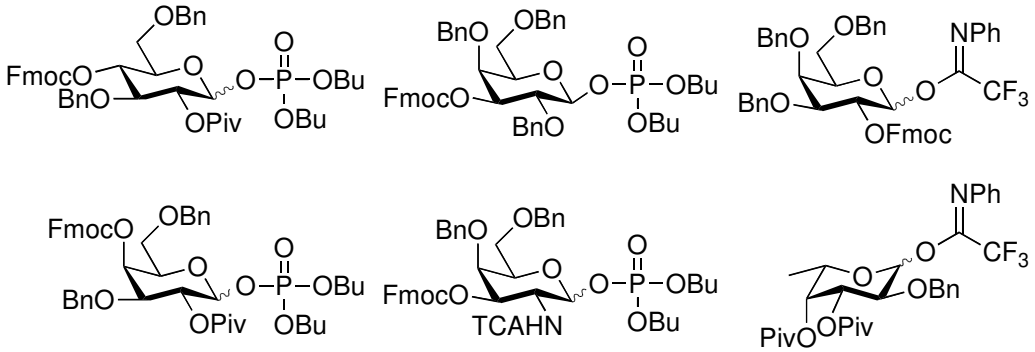
Globo-H: Retrosynthesis



Globo-H Hexasaccharide

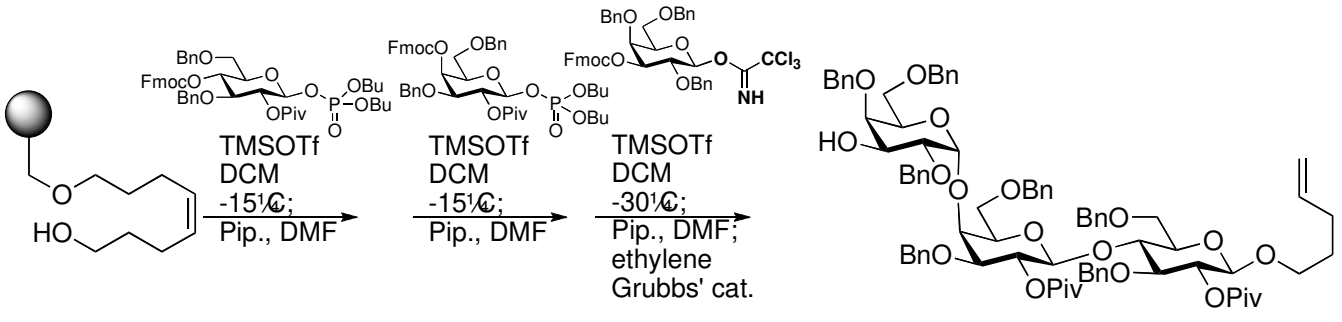


Gb3 Trisaccharide



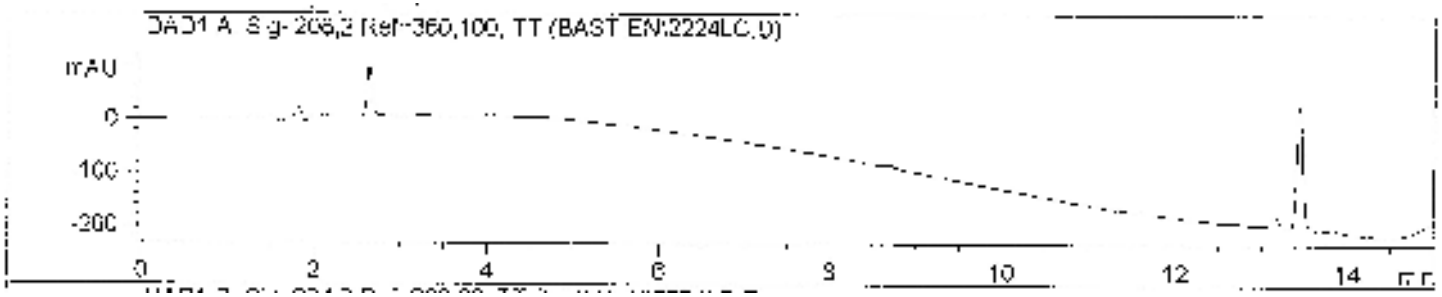
Werz, D. B.; Castagner, B.; Seeberger, P. H. *J. Am. Chem. Soc.* 2007, *129*, 2770

Gb3 synthesis

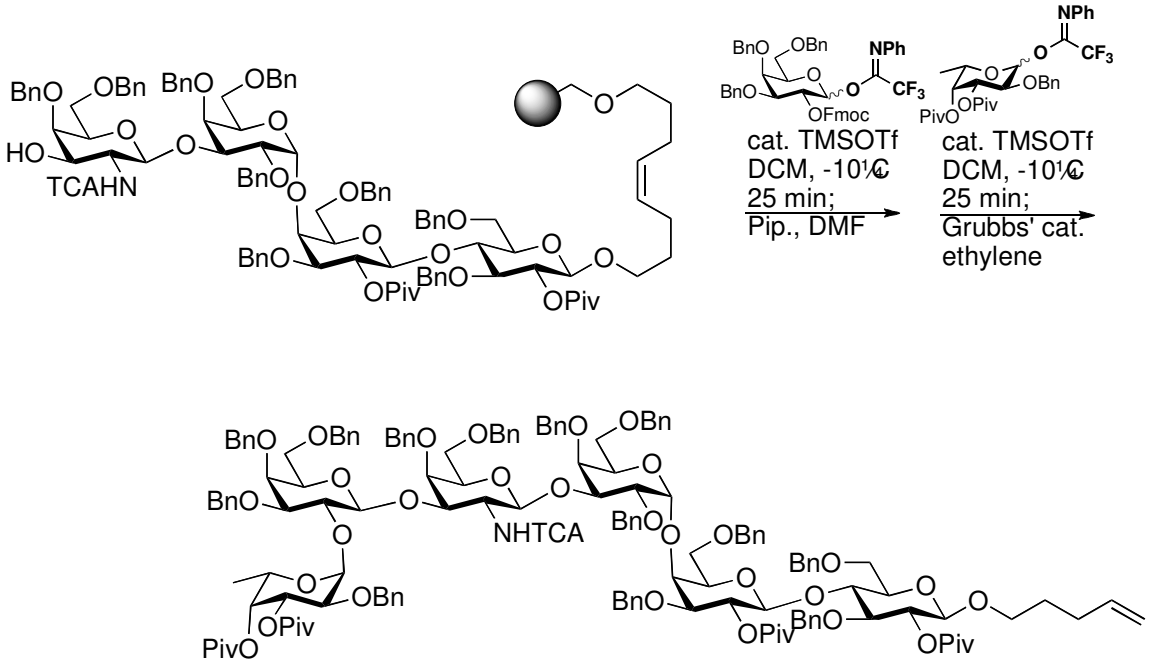


Gb3
46% isolated yield

Crude HPLC

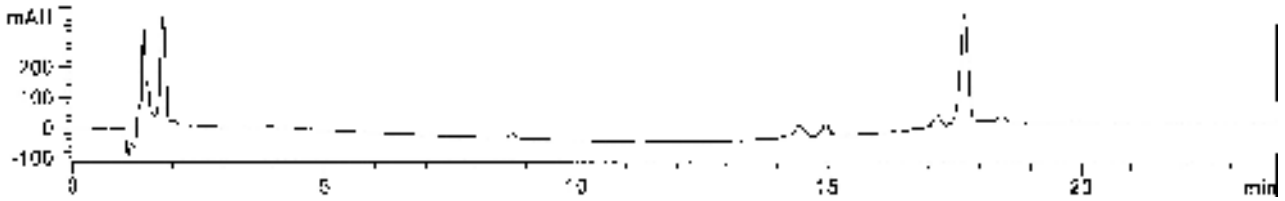


Globo-H Synthesis

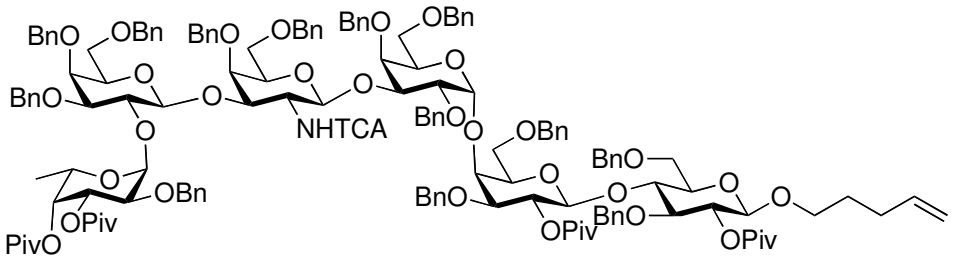


Globo-H Hexasaccharide

Crude HPLC

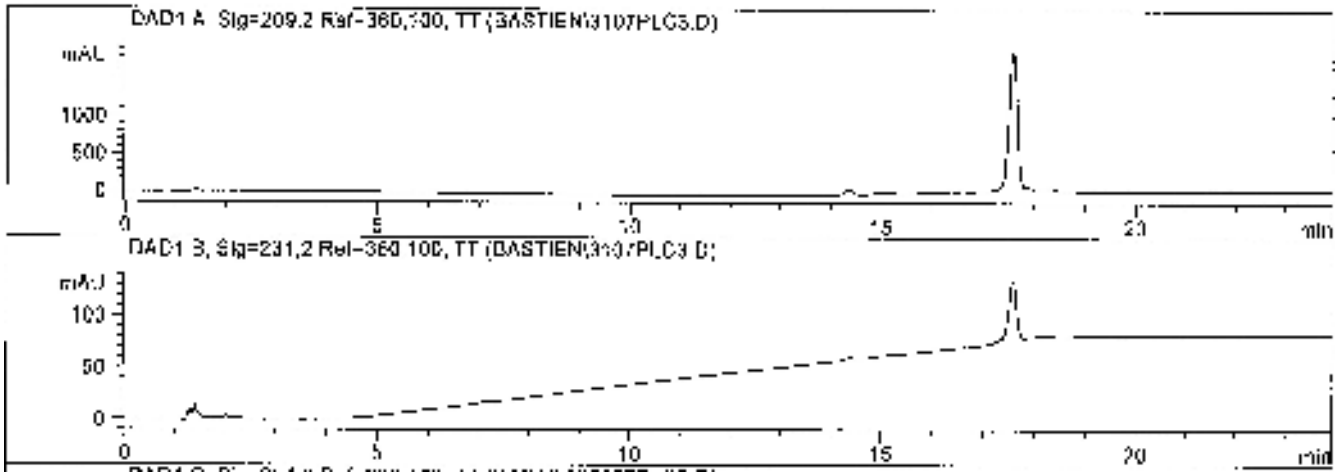


Purified Globo-H



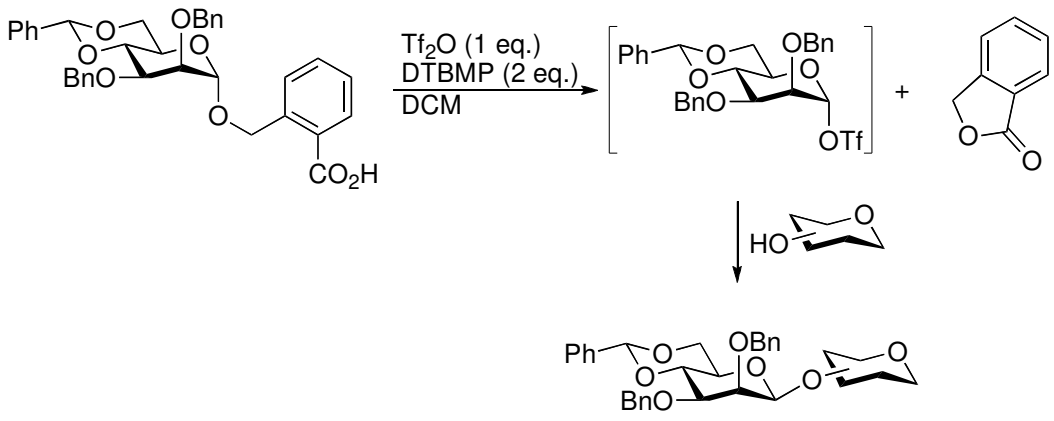
Globo-H Hexasaccharide

30% overall yield after column chromatography



Werz, D. B.; Castagner, B.; Seeberger, P. H. *J. Am. Chem. Soc.* 2007, *129*, 2770

β-Mannosylation



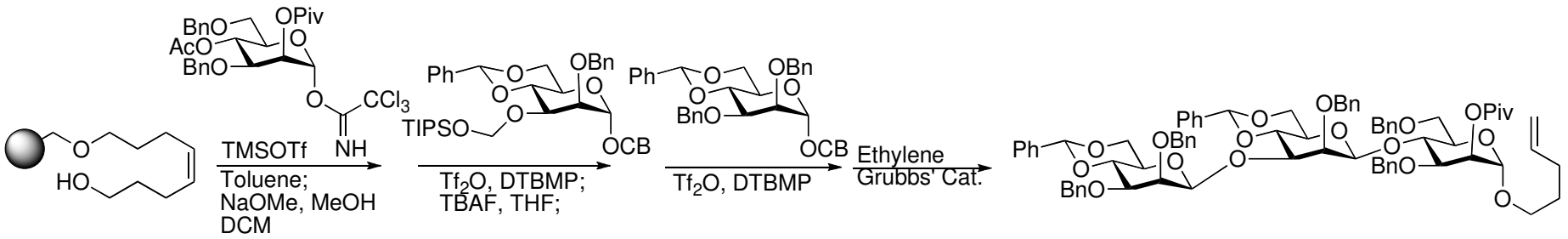
-No pre-activation necessary

-Compatible with linker olefin

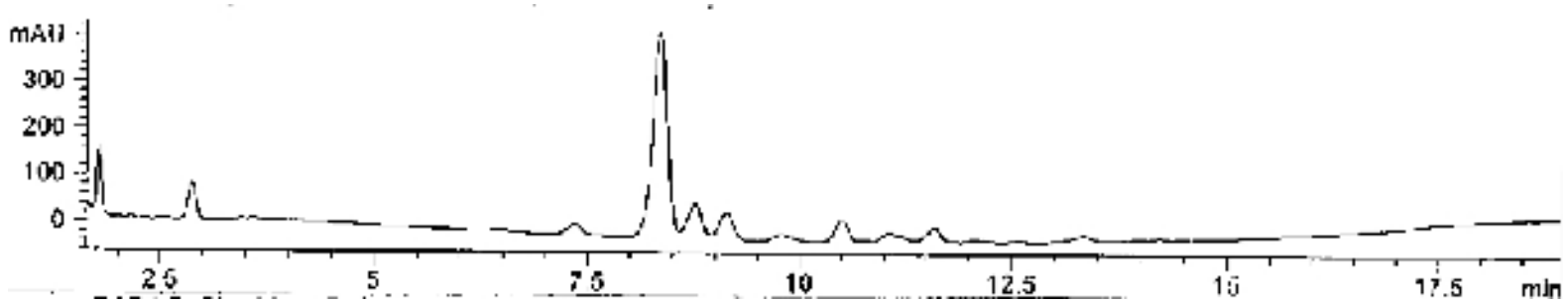
-No acceptor by-product formation

Kim, K. S.; Kim, J. H., Lee, Y. J.; Lee Y. J.; Park, J. *J. Am. Chem. Soc.* 2001, *123*, 8477-8481

Elongation of the C3 Position

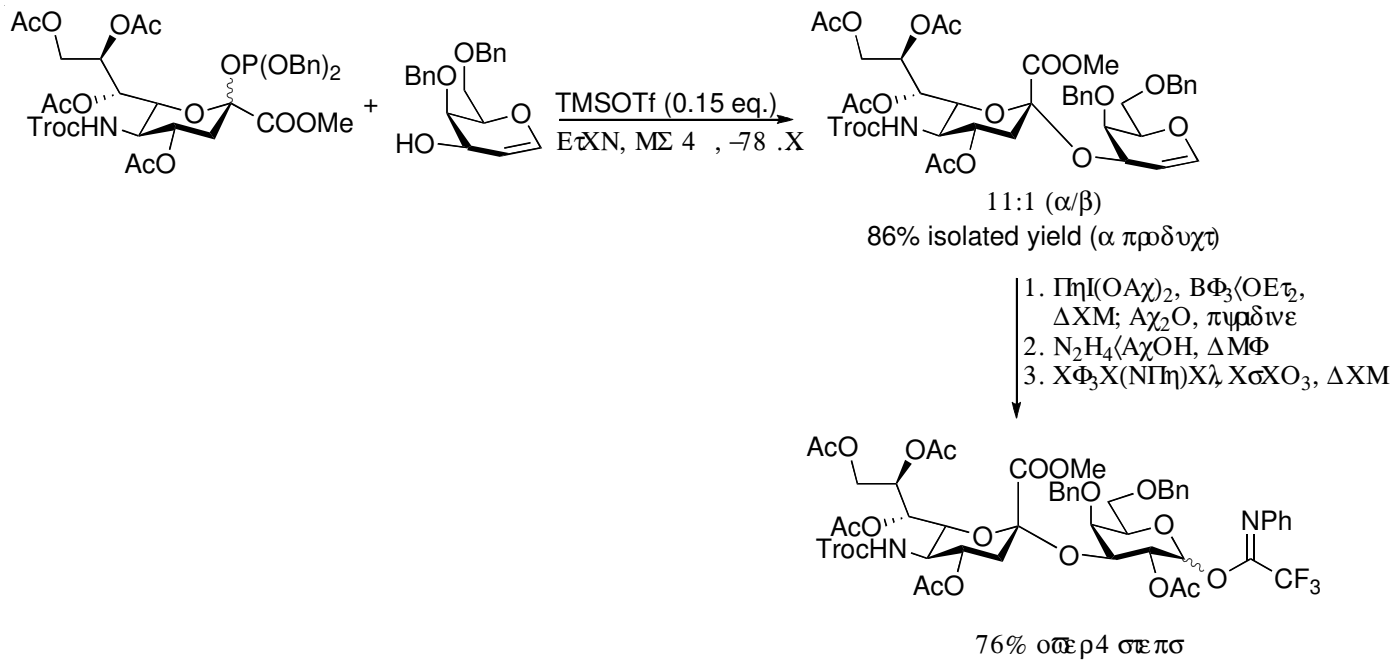


60% isolated yield (over 6 steps)



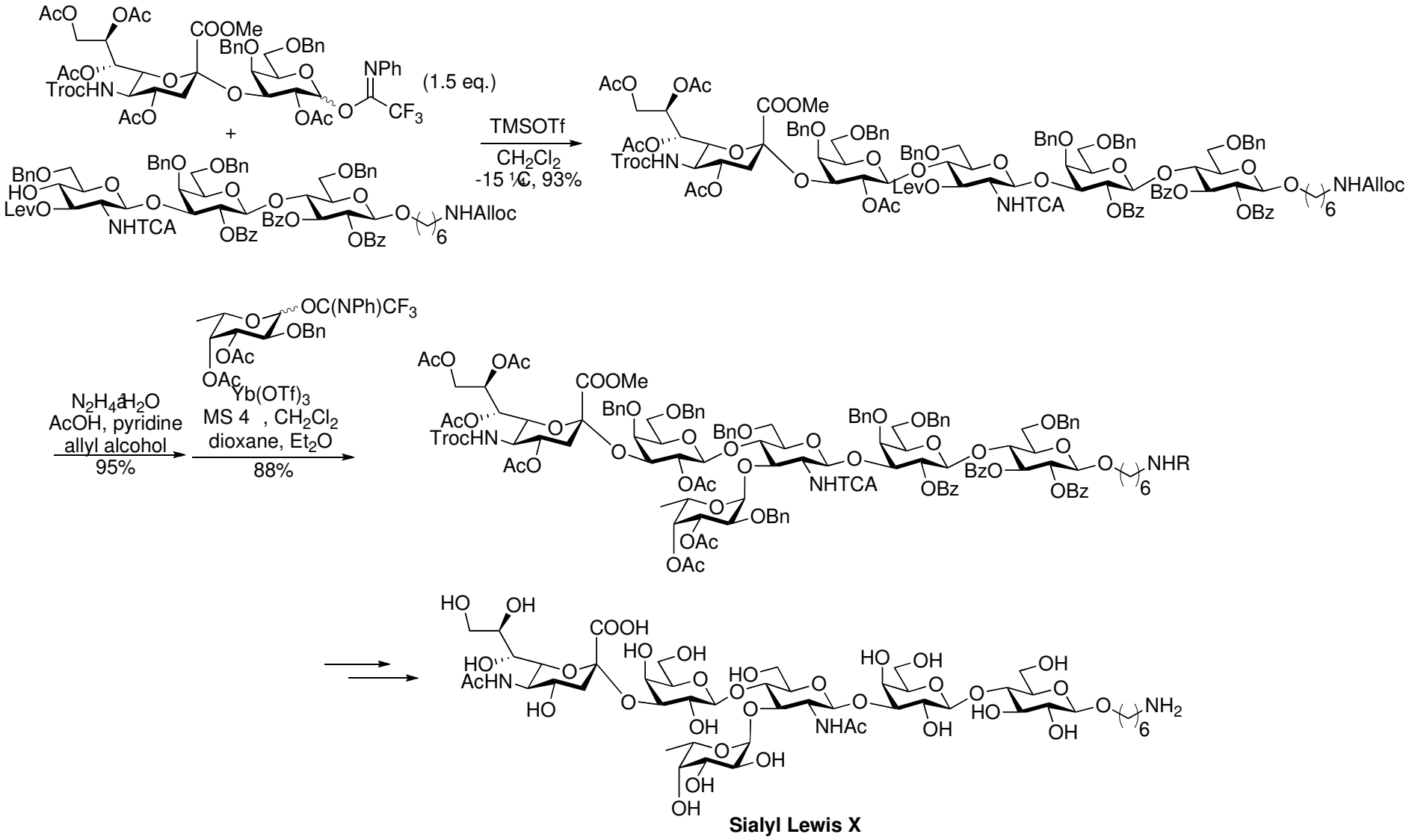
Codée, J. D. C.; Kröck, L.; Castagner, B.; Seeberger, P. H. *in preparation*

Sialic Acid Disaccharide Building Block

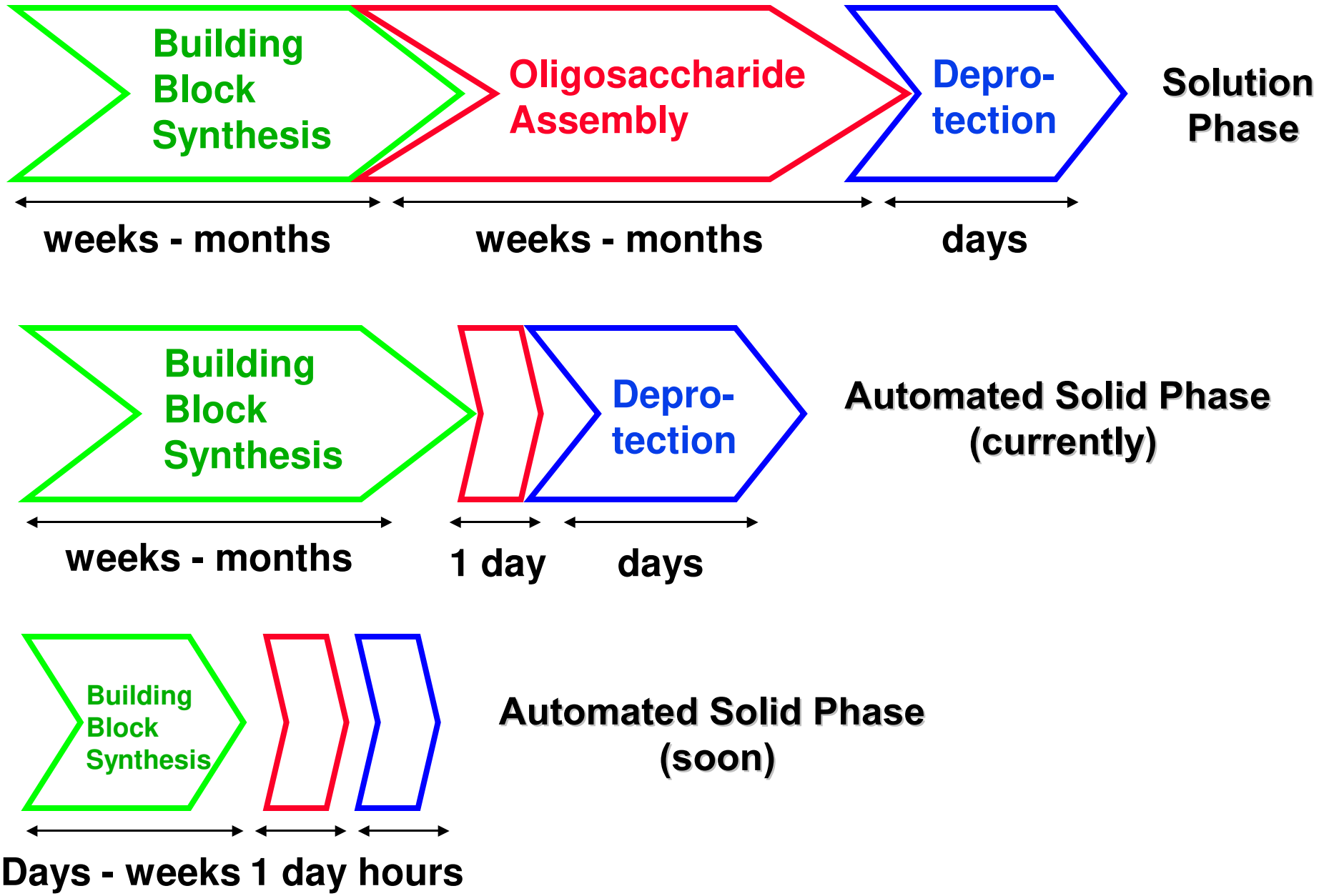


Hanashima, S.; Castagner, B.; Esposito, D., Nokami, T.; Seeberger, P. H. *Org. Lett.* 2007, *9*, 1777.

Solution Phase Sialyl Lewis X Synthesis



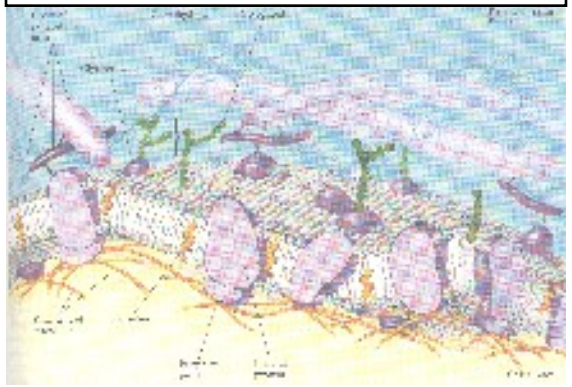
Time Allocation During Oligosaccharide Synthesis



Development of Vaccine Candidates Against Parasites, Bacteria and Cancer

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

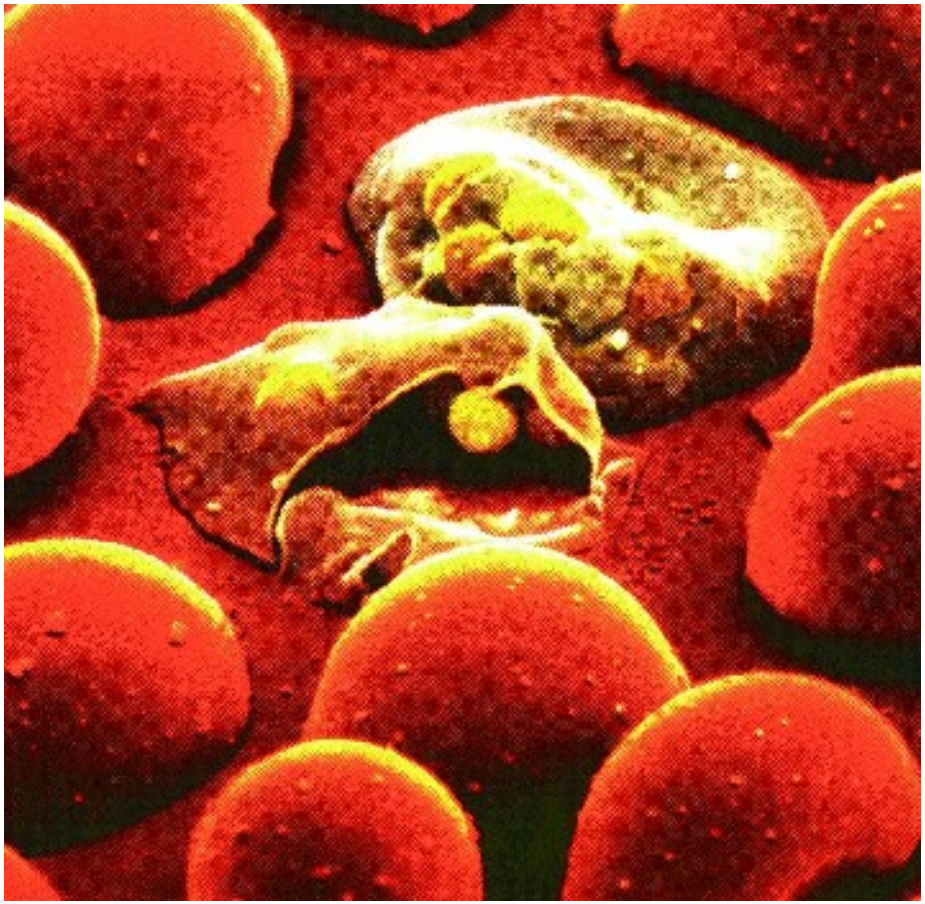
Preclinical Development

Malaria

ancora

Clinical Development

An Anti-Toxin Malaria Vaccine



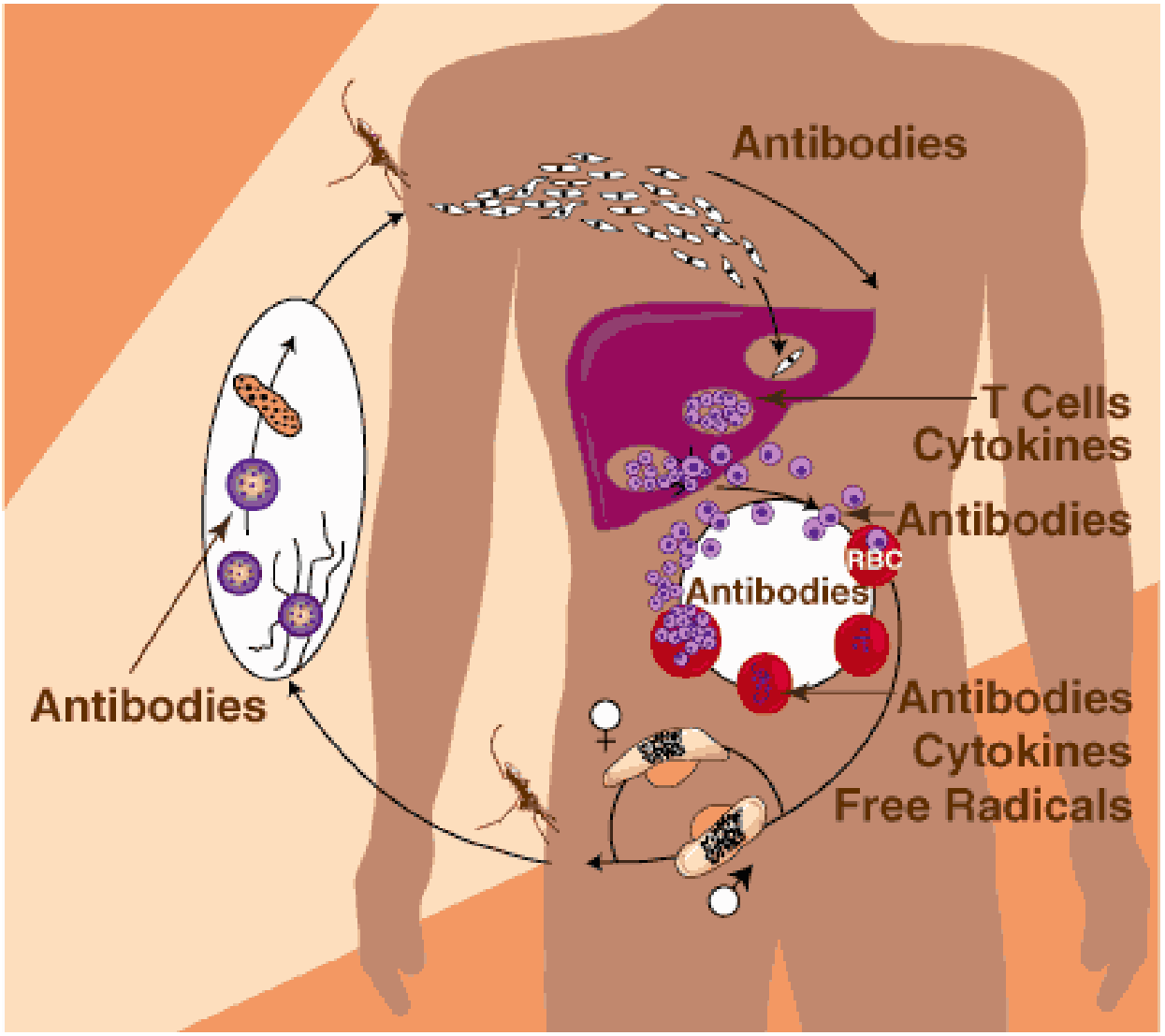
Clinical and Anti-parasite Immunity to Malaria

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

QuickTime[a] and a
Photo - JPEG decompressor
are needed to see this picture.

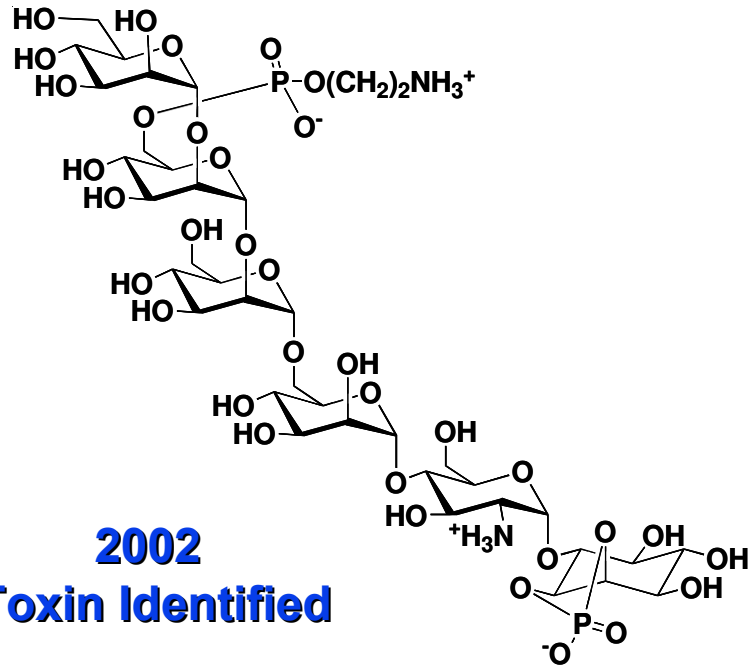
Malaria Statistics (1994 WHO Estimate)
- 40% of world population at risk
- 5% infected (300 million people)
- 100 million clinical cases
- 2-3 million deaths (1% of cases fatal
  (predominantly children < 5 years)

The *Plasmodium falciparum* Life Cycle



An Anti-Toxin Malaria Vaccine

1896
Golgi Postulates Malaria Toxin



2002
Toxin Identified

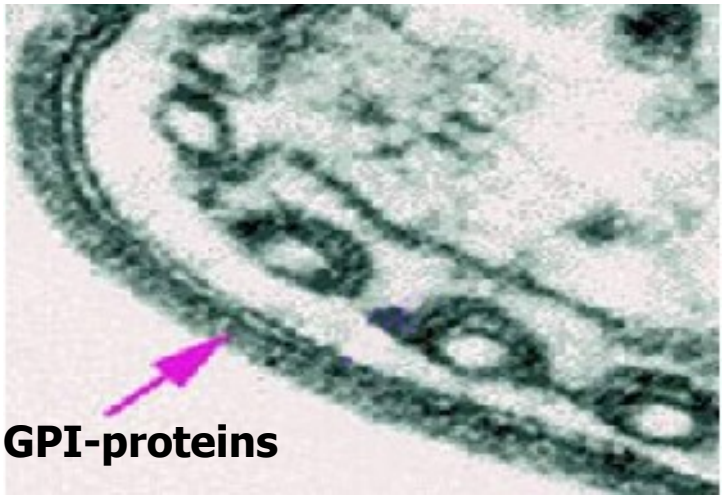
1) Substance isolated from *P. falciparum* - structure postulated

2) Synthesis of structure to confirm assignment

3) Use synthetic molecule as anti-toxin vaccine candidate

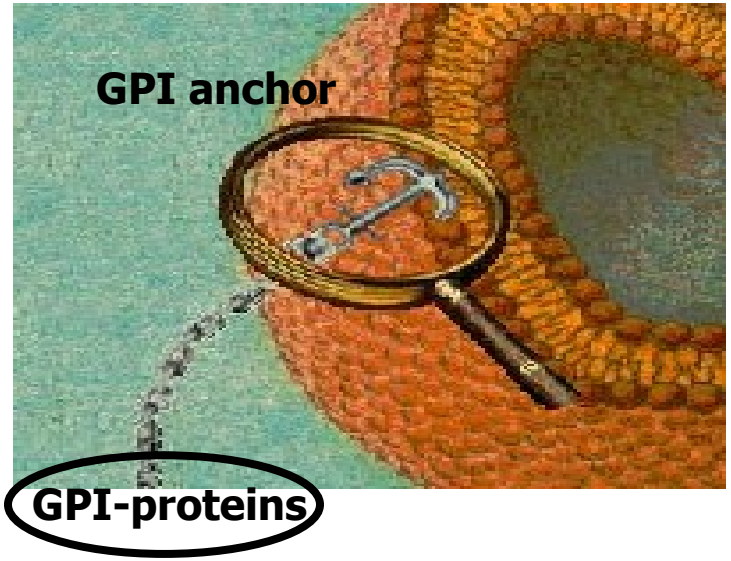
Nature, 2002, *418*, 785

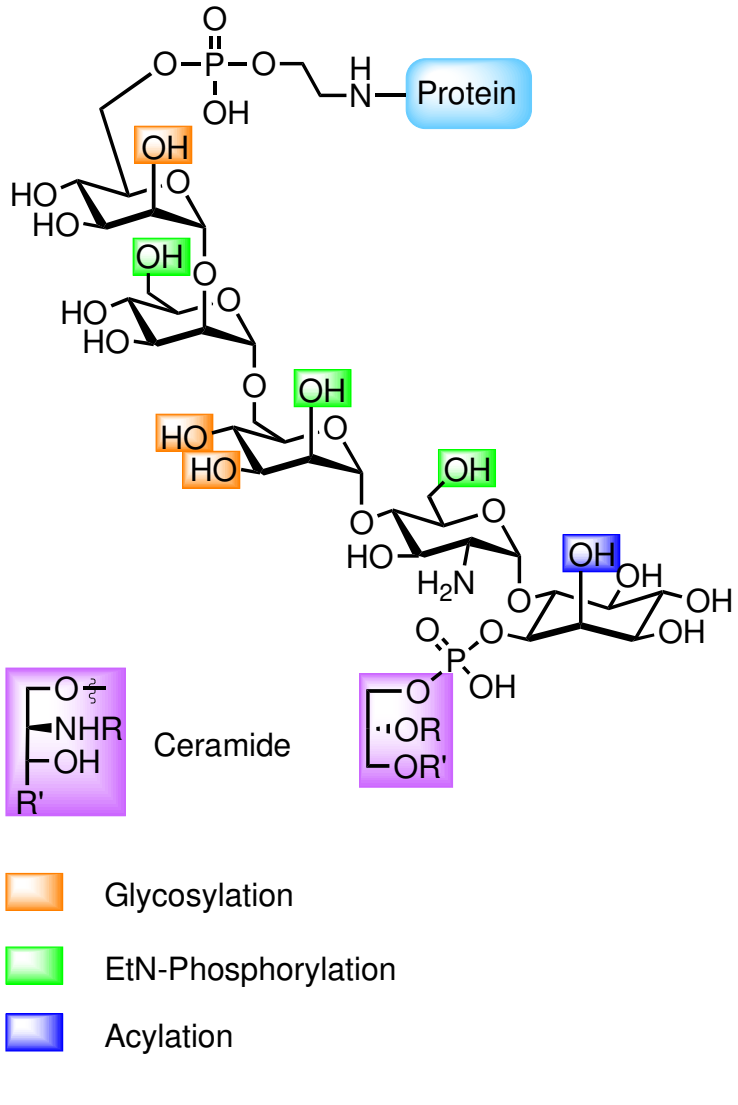
Glycosyl Phosphatidyl Inositol (GPI): Structure



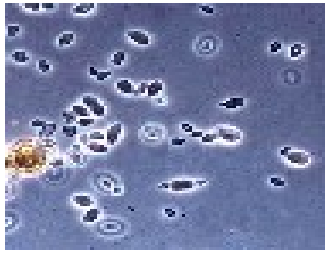
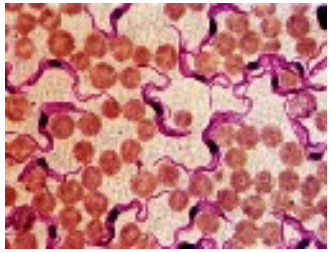
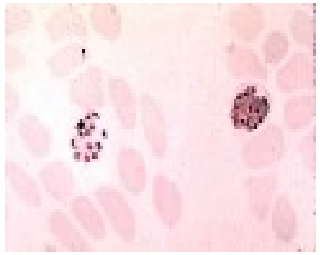
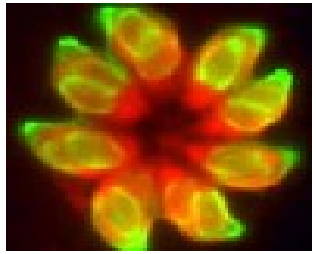
>GPI-proteins

>Free GPIs





Glycosyl Phosphatidyl Inositol (GPI): Occurrence


Yeast *T. brucei* *P. falciparum* *T . gondii* Human

High copy (10-20 Million per cell) Low copy

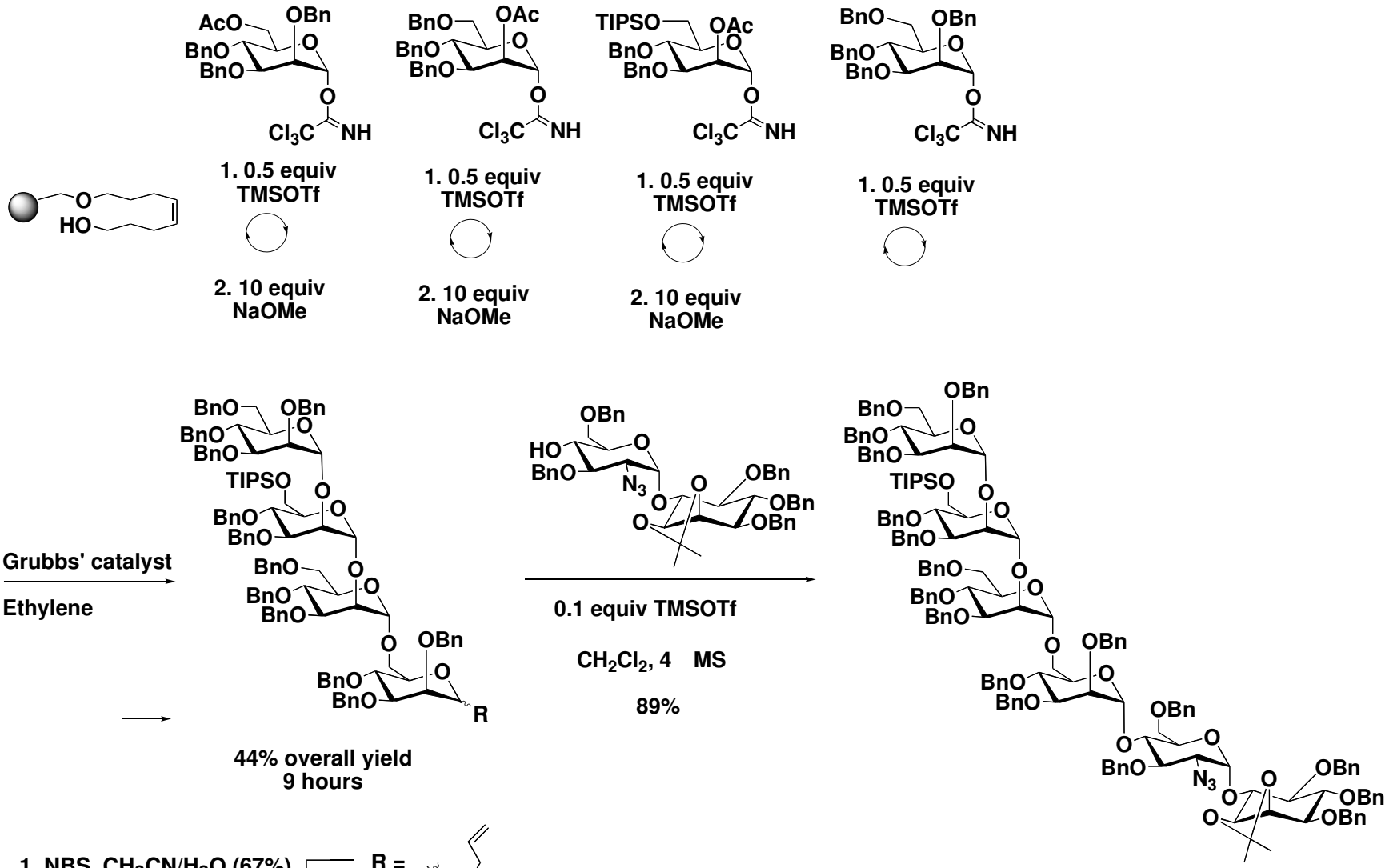
Exoenzymes e.g. alkaline phosphatase,

Adhesion molecules e.g Neural cell adhesion molecules

Complement regulatory proteins e.g. DAF, CD59

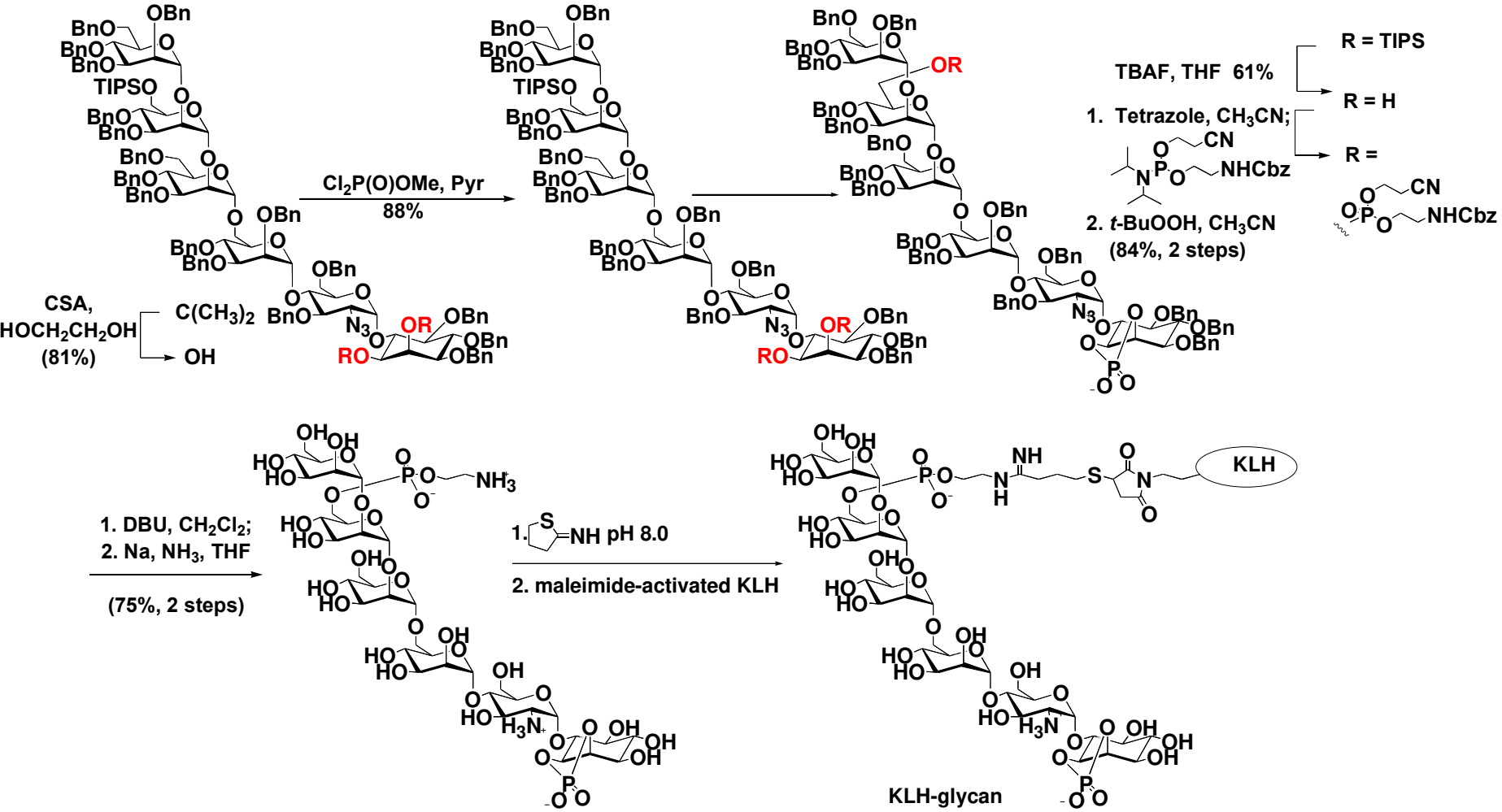
Protozoa surface antigens e.g. SAG1, MSP1

Semi-Automated Assembly of the GPI Glycan



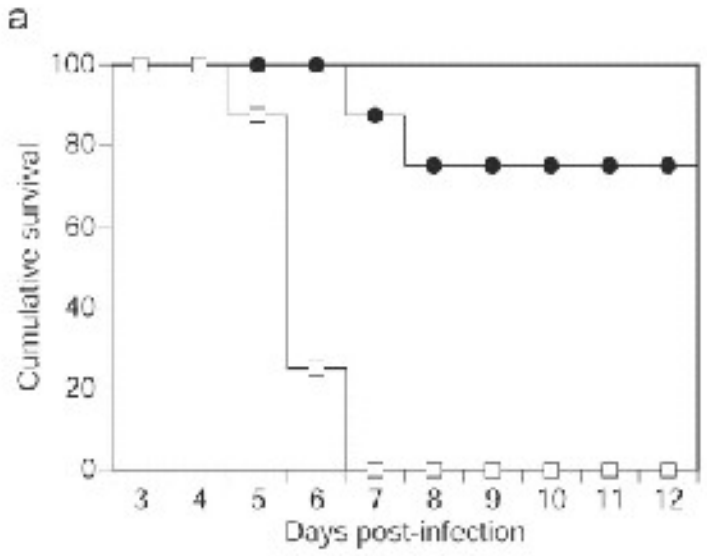
J. Am. Chem. Soc. 2002, *124*, 13434.

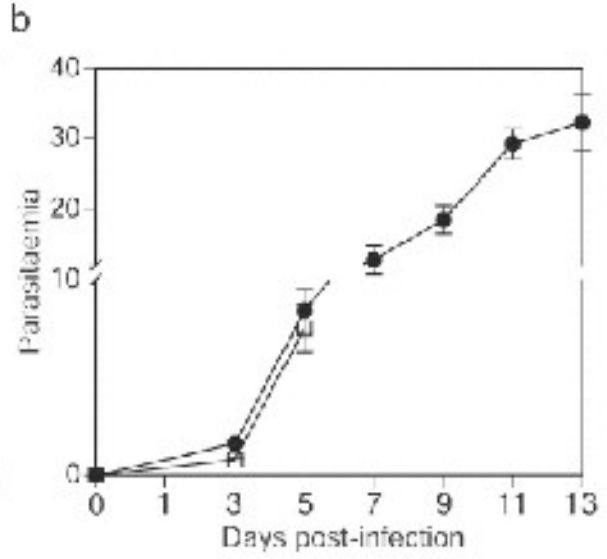
Synthesis of a Malaria Vaccine Candidate



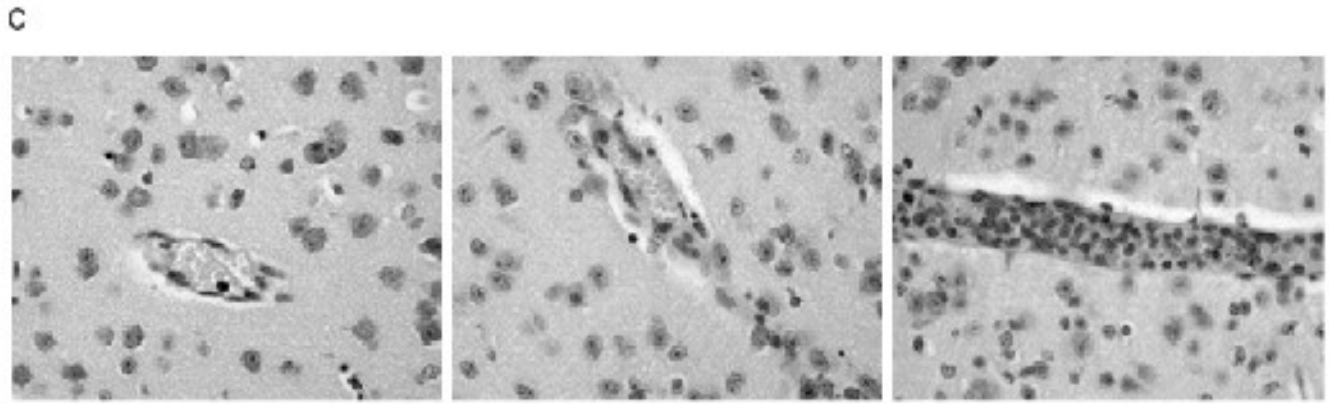
Nature, 2002, *418*, 785

Vaccines vs Controls

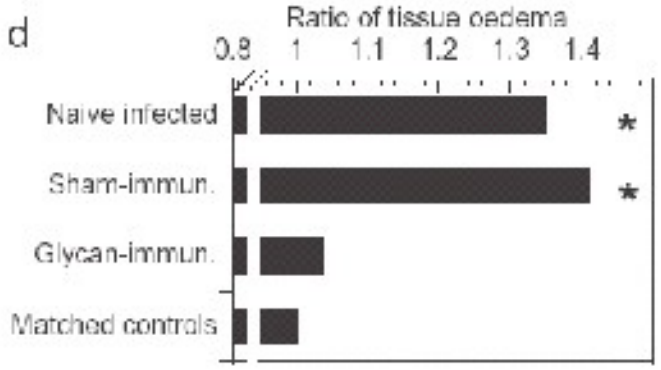


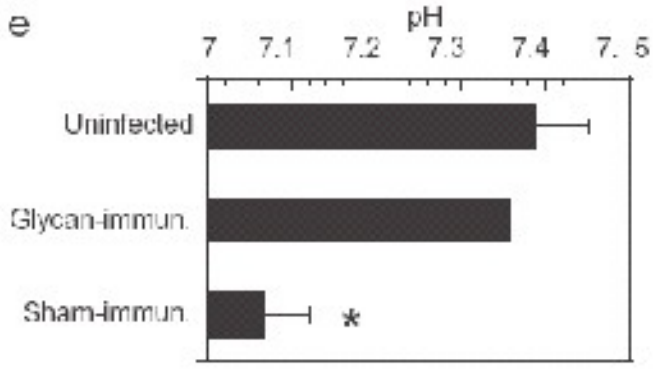


Survival and parasitaemia



Cerebral Histology

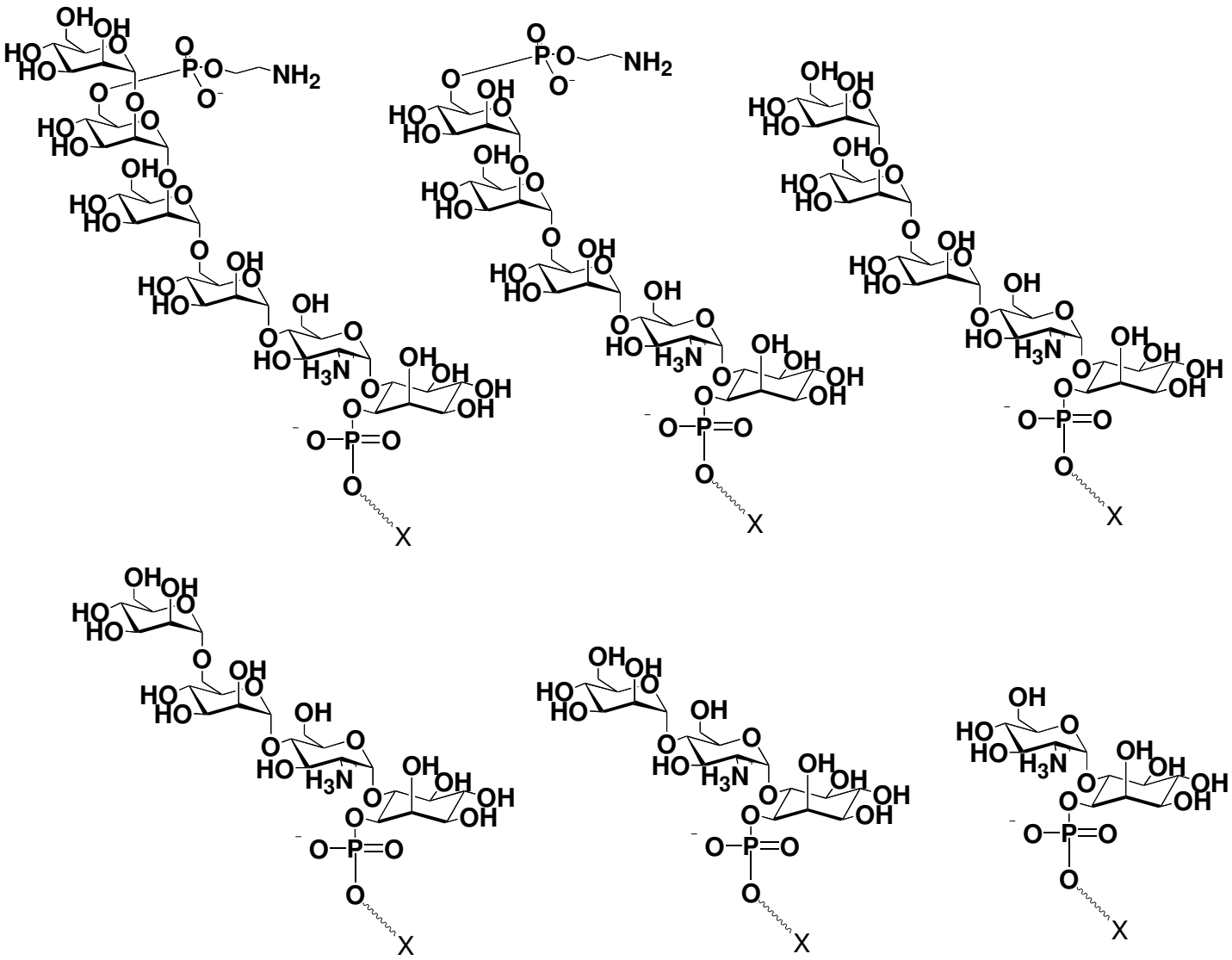




Systemic Pathology

Does an Anti-GPI Response Protect from Malaria Mortality?

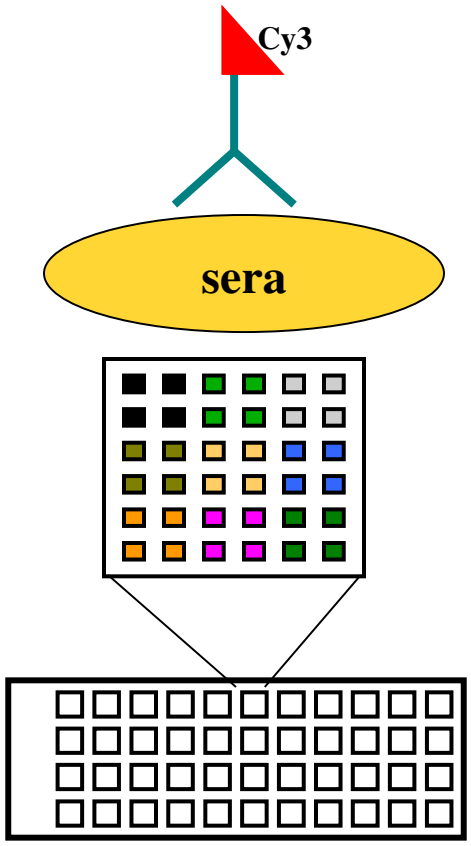
Tools for Epitope Mapping & Biosynthesis Investigations

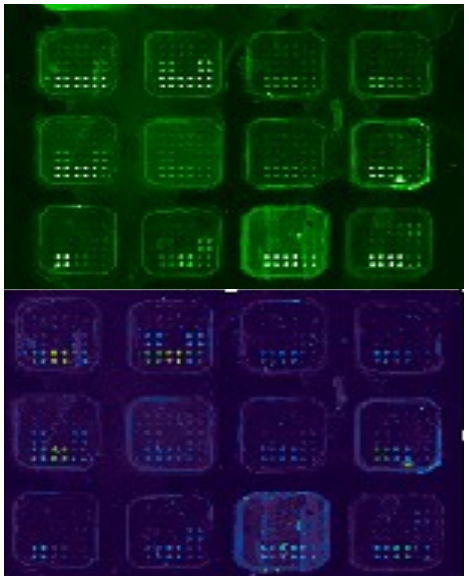


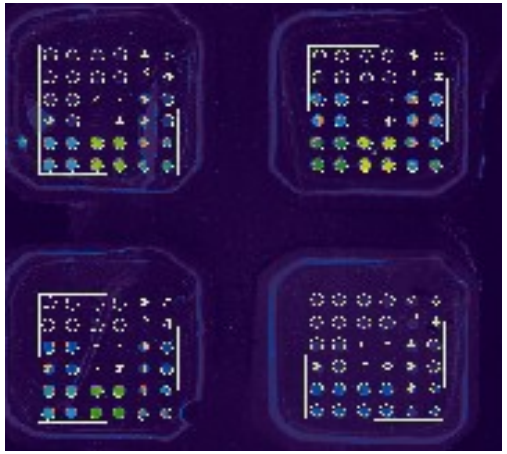
Chem. Eur. J. 2005, *11*, 2493.

High Throughput Detection of Anti-GPI Antibodies on Microarrays

Incubation → **Read Out** → **Quantification**



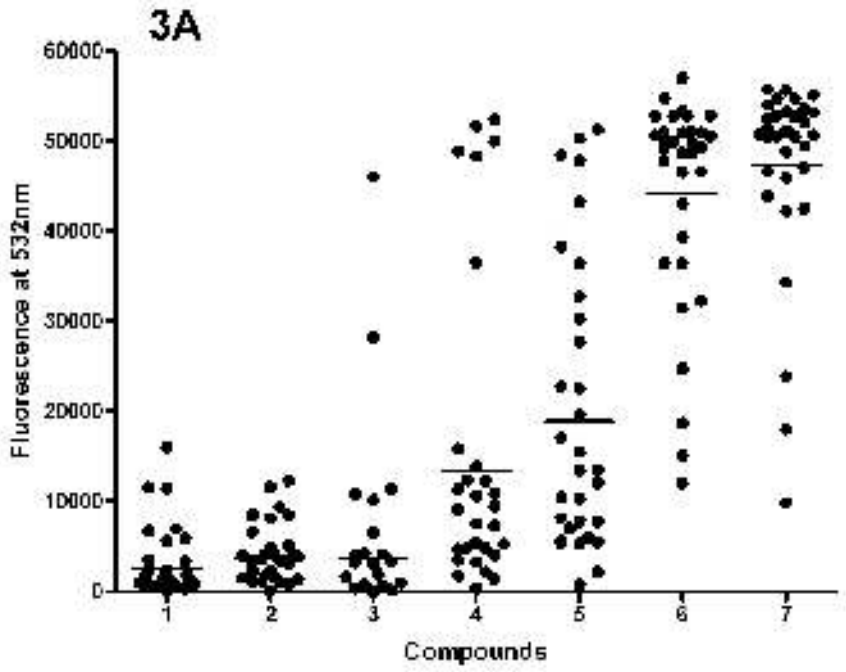




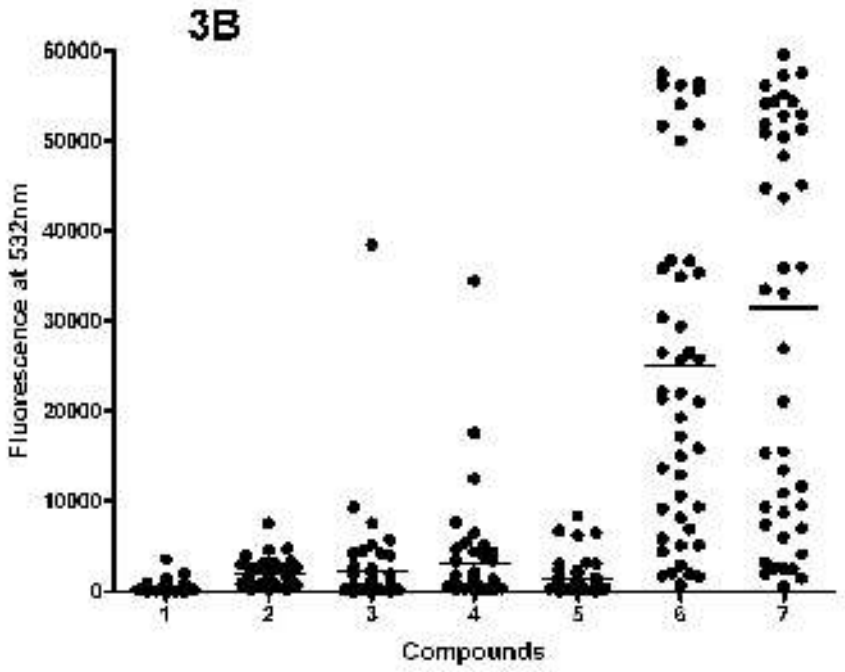
Kamena, J.F.; Tamborrini, M.; Liu, X.; Pluschke, G.; Seeberger, P.H. submitted

Mean Anti-GPI IgG Titres in Human Sera

Malaria endemic area of Burkina Faso



Malaria non-exposed Europeans



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

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

S. Bufali
X. Liu
C. Noti
L. Hossein
A. Adibekian
L. Kroeck
K. Geyer
R. Castelli
P. Bindschädler
T. Horlacher
M. Oberli
P. Seif
D. Esposito
Y. Guo
P. Stallforth

Dr. F. Kamena
Dr. N. Azzouz
Dr. F. Carell
Dr. B. Castagner
Dr. S. Hanashima
Dr. R. Wada
Dr. H. Wippo
Dr. Boonyarattanakalin
Dr. T. Gustafsson
Dr. R. Gilmour
Dr. K. Raghavendra

The Burnham Institute

Dr. P. Wang
Dr. F. Wallner
Dr. S. Takashima

Collaborations

Dr. Becker
Prof. Schofield
Prof. Jensen
Prof. Textor
Prof. Pluschke
Prof. Chatterjee
Prof. Schachner
Prof. Hengartner

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DFG (Emmy Noether Program)
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