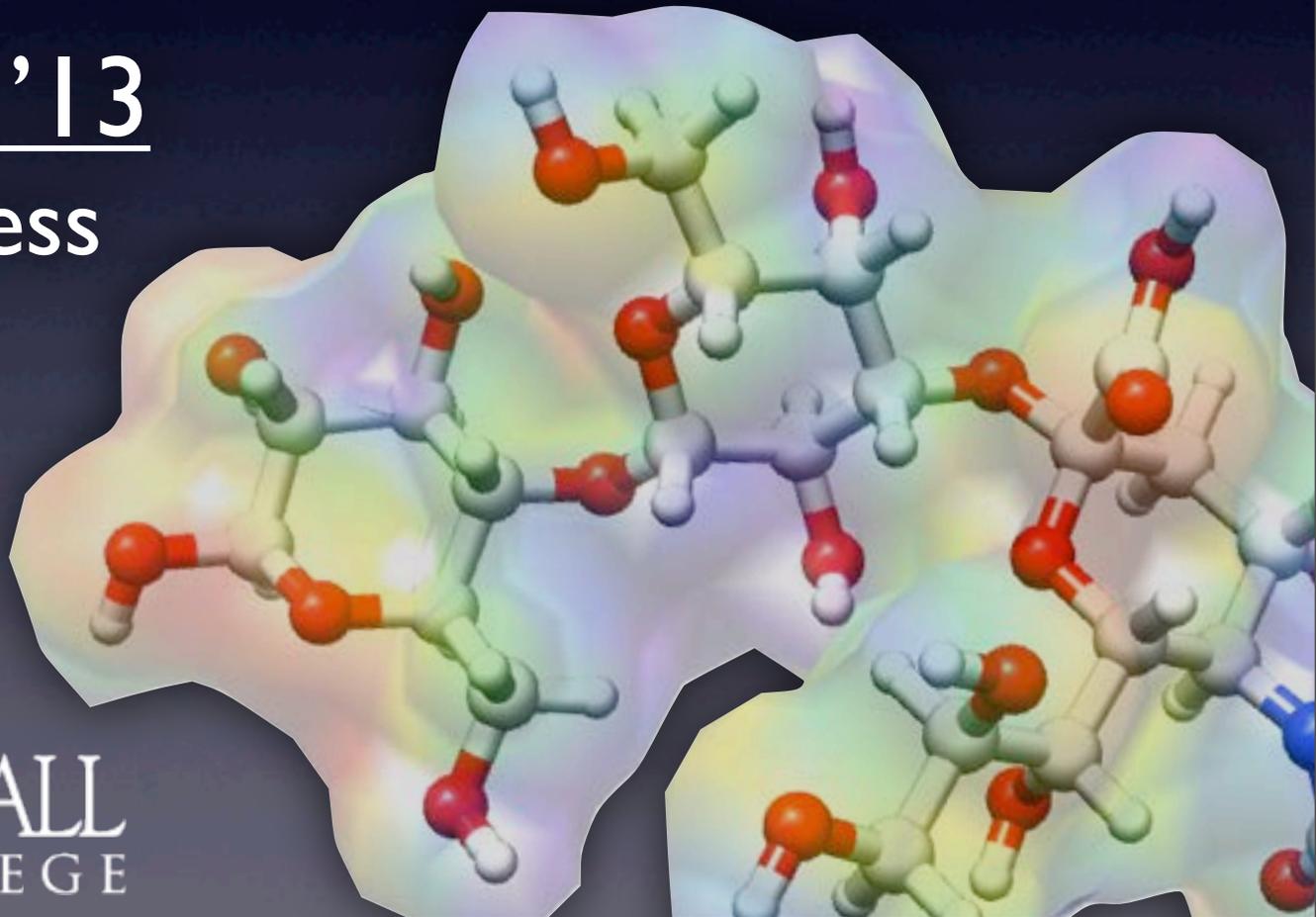


# Highlight: GM3 Synthase Deficiency

---

Joshua Wesalo '13

Advisor: Ken Hess



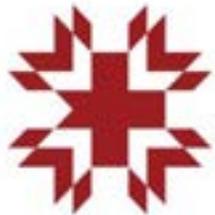
“Better living through  
chemistry”

# Collaborations—The GM3 Team



THE  
UNIVERSITY OF  
BRITISH  
COLUMBIA

Dr. Steve Withers



Clinic for Special Children



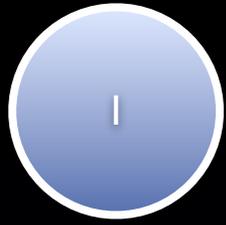
## Drug Development Consultants

Dr. Steve Roth

Dr. David Zopf

Dr. Matt Kremer

- Dr. Ken Hess
- Dr. Scott Brewer
- Dr. Ed Fenlon
- Dr. Rob Jinks
- Dr. Scott Van Arman
- Dr. Christine Piro



What is GM3?



Synthetic Strategy



Results so far



Future Directions

# GM3 Synthase Deficiency

Founder effect in Amish leads to...

– Blindness —

– Epilepsy —

– Deafness —

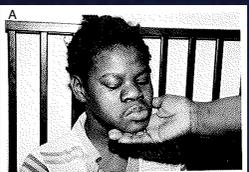
– Developmental Stagnation —

- Can't walk, talk, reach, or sit
- Nonpurposeful movements
- Muscular weakness
- Brain atrophy



# Patients

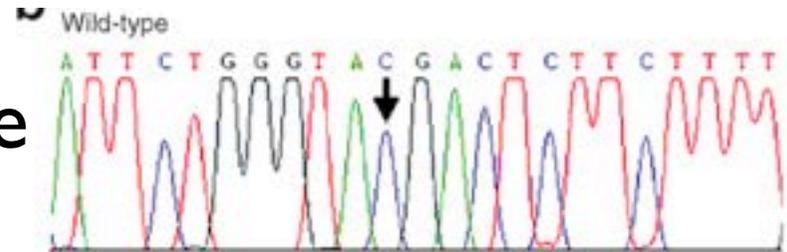
1 in 100 are carriers



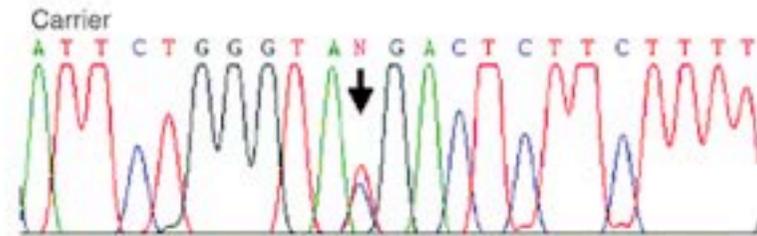
# Genetics & Disease Mechanism

Nonsense mutation in GM3 Synthase (SIAT9) gene  
(694C→T, translating to R232X)

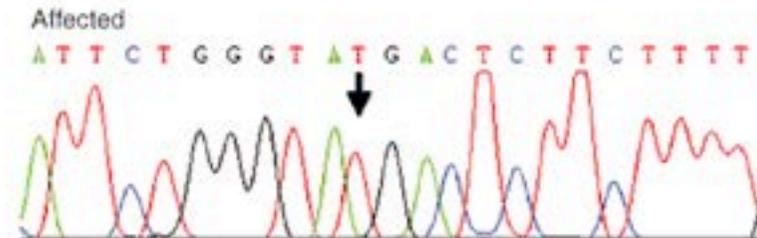
Wild-Type



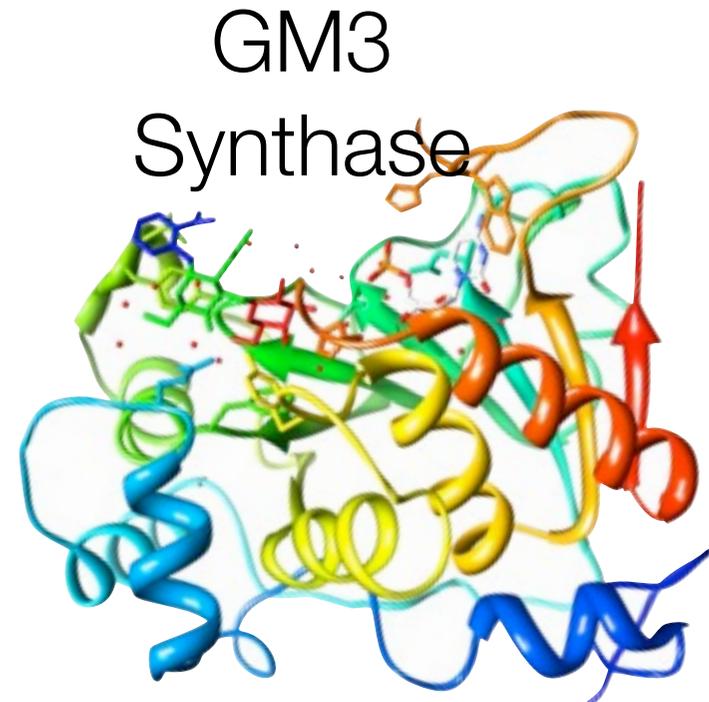
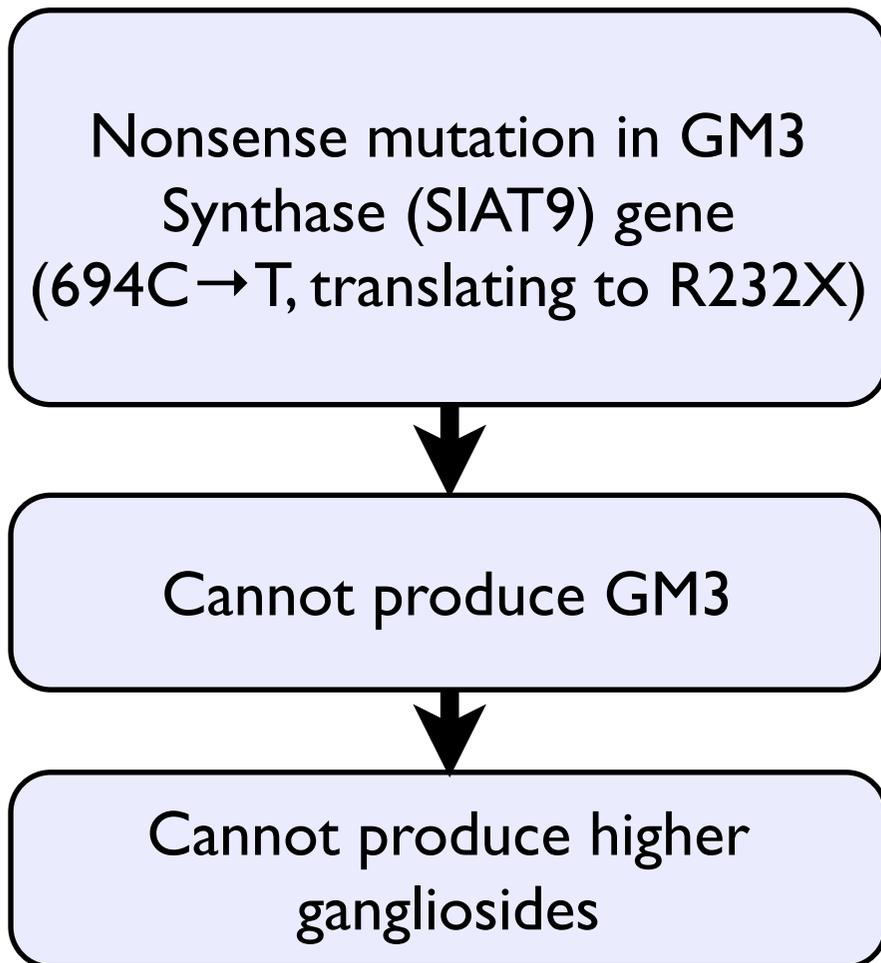
Carrier



Affected



# Genetics & Disease Mechanism



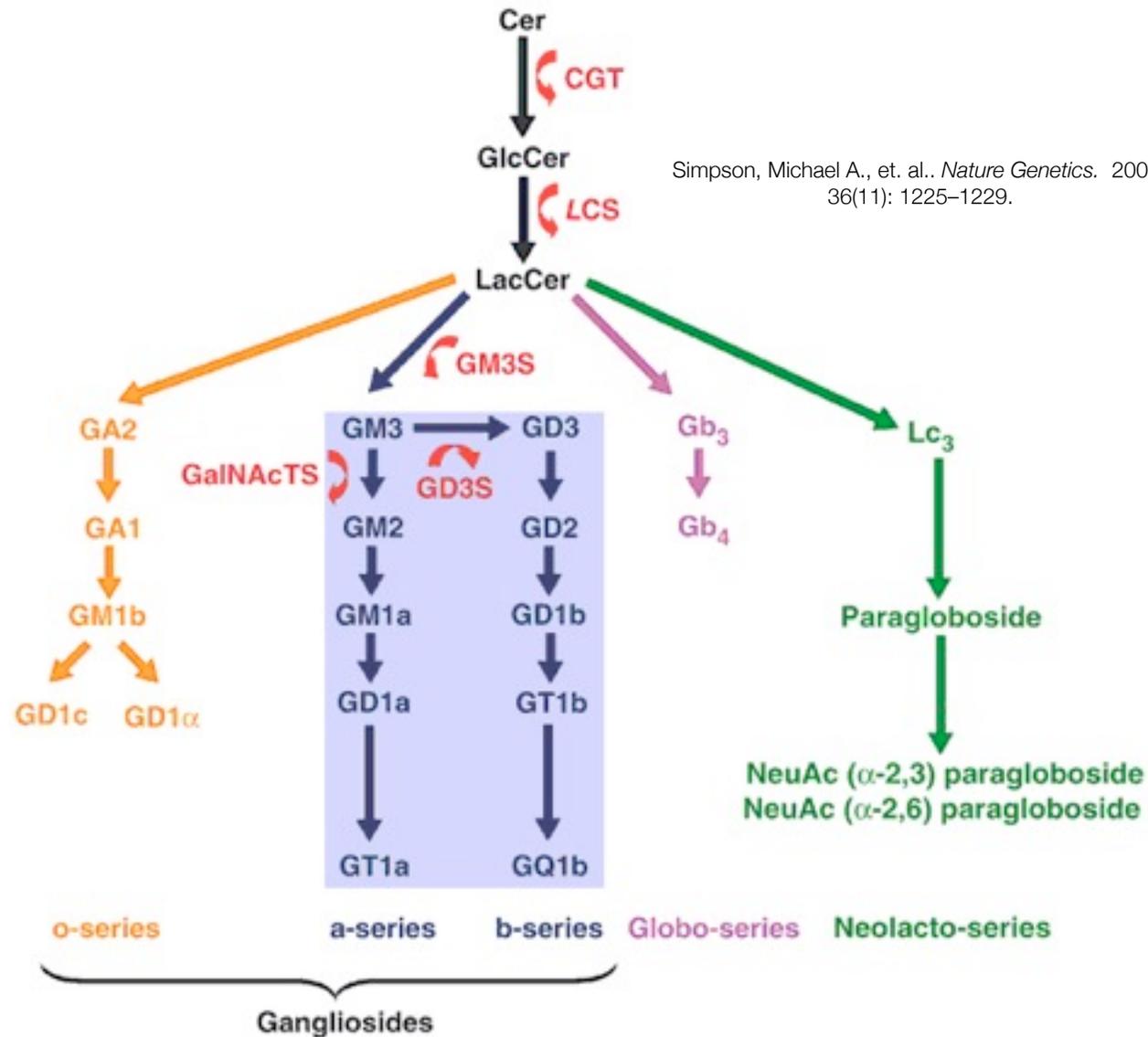
Adapted from Rao FV et. al.. *Nature Structural & Molecular Biology*. 2009; 16: 1186–1188.

Problems in cellular adhesion<sup>1</sup>, cell signaling<sup>1</sup>, and axon-glia interactions<sup>2</sup>

1-Simpson, Michael A., et. al.. *Nature Genetics*. 2004; 36(11): 1225–1229.

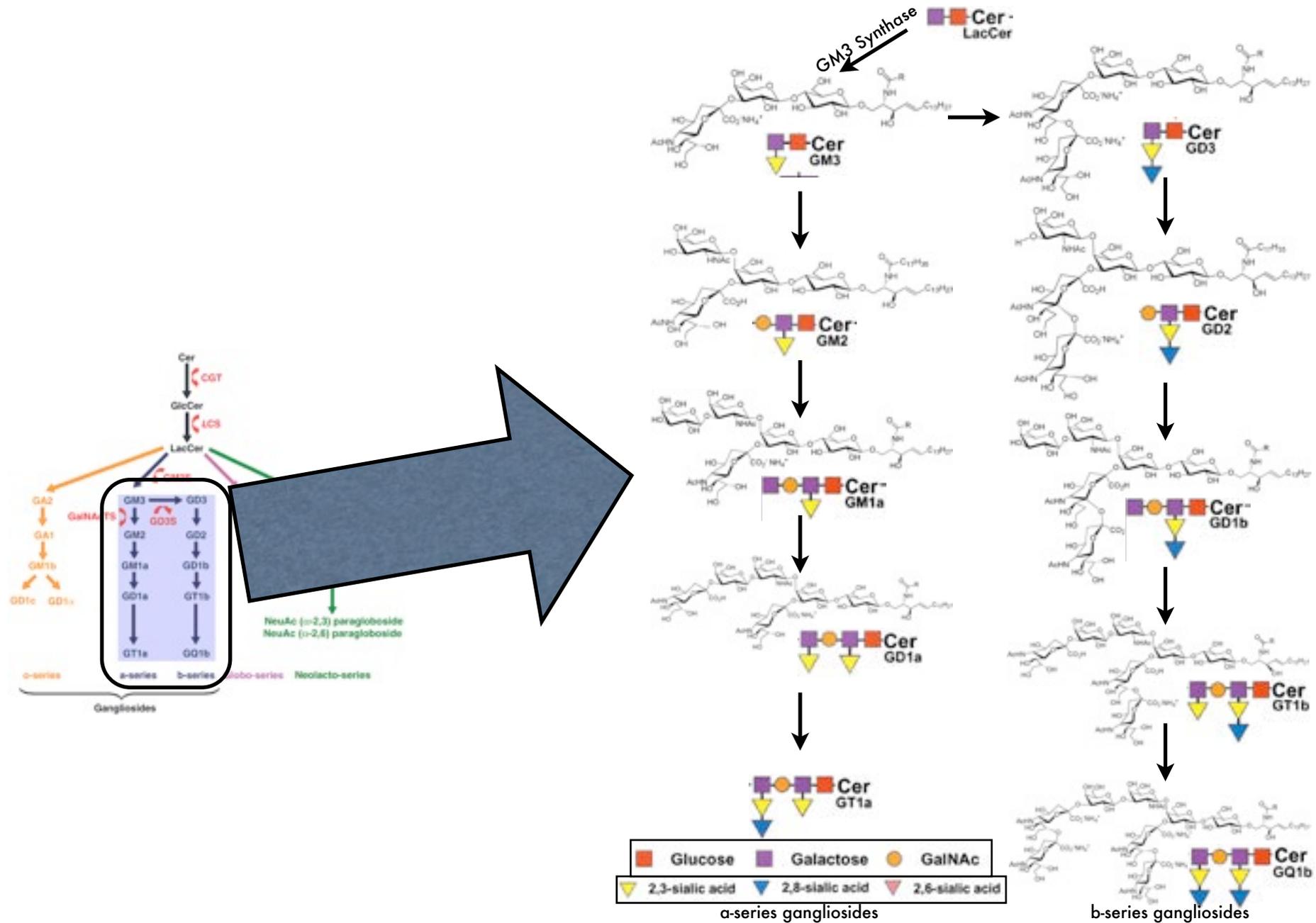
2-Yamashita, Tadashi, et. al.. *PNAS*. 2005; 102(8): 2725–2730.

# Genetics & Disease Mechanism



Simpson, Michael A., et. al. *Nature Genetics*. 2004; 36(11): 1225-1229.

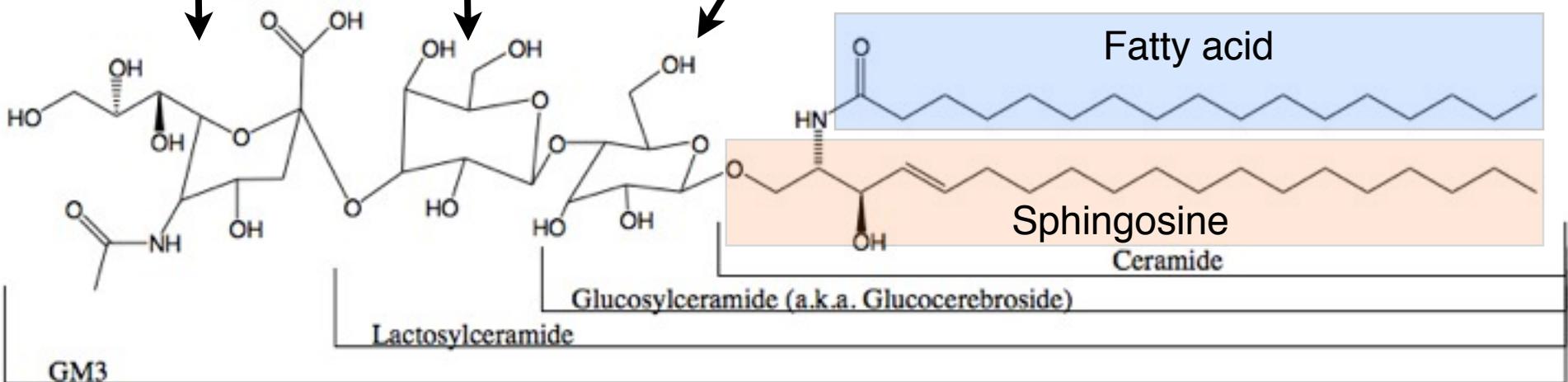
# Genetics & Disease Mechanism



# GM3

pK<sub>a</sub> 2.2–3.0

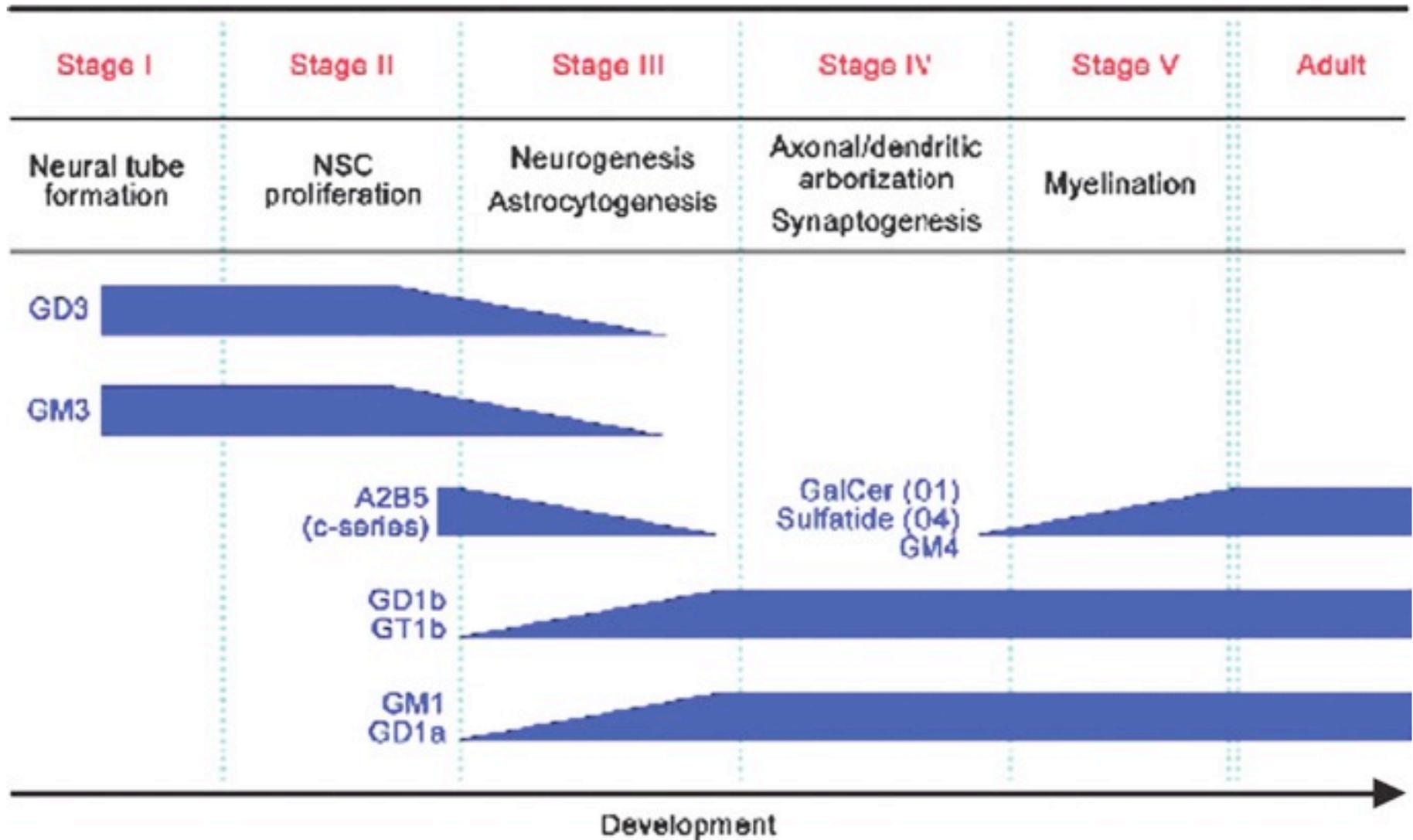
Neu5Ac Galactose Glucose



Neu5Ac( $\alpha$ 2 $\rightarrow$ 3)Gal( $\beta$ 1 $\rightarrow$ 4)Glc( $\beta$ 1)Cer

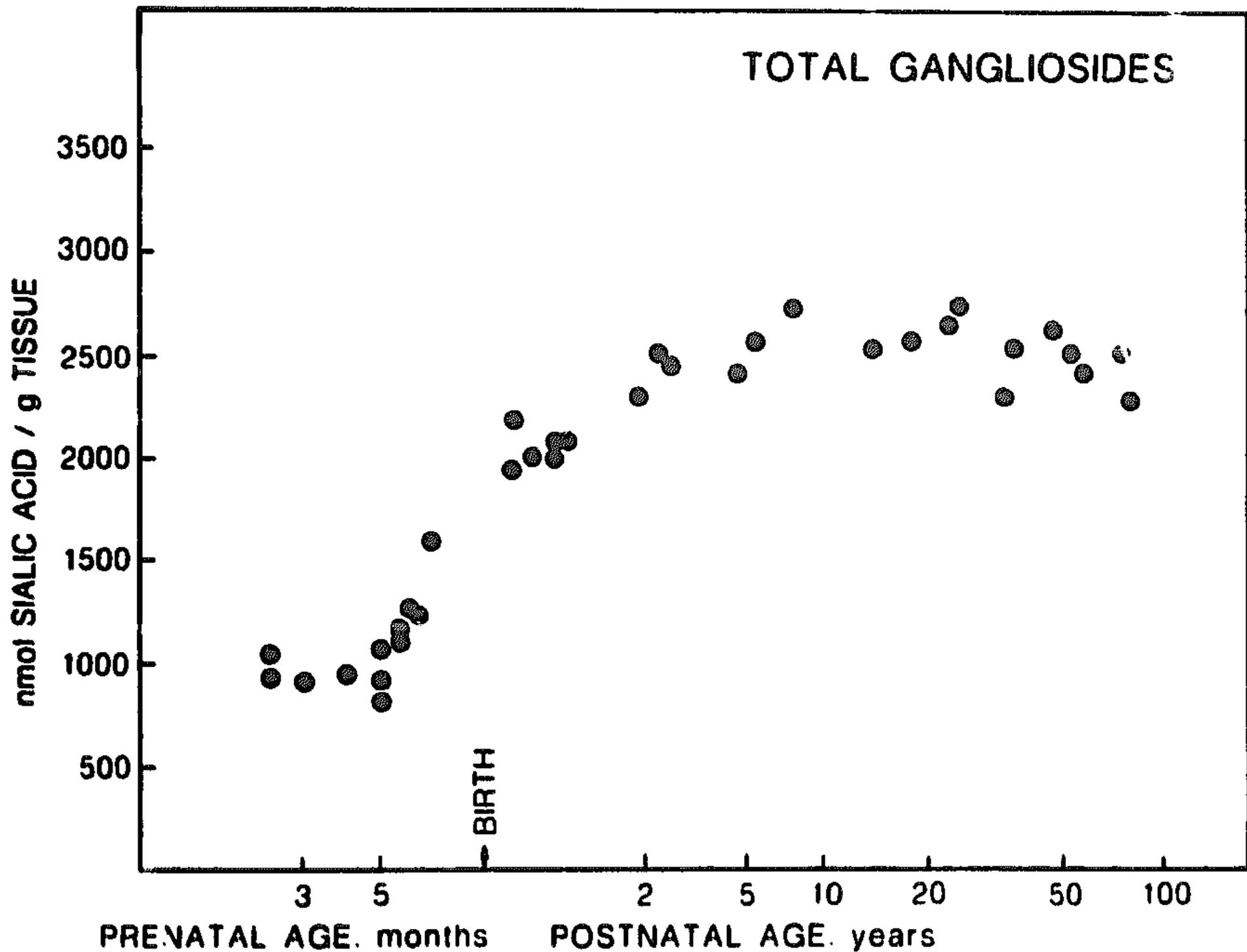
$10^{-7}$  M > CMC >  $10^{-9}$  M (in aqueous solution)

## Neurodevelopmental milestones and concurrent changes in GSL expression.

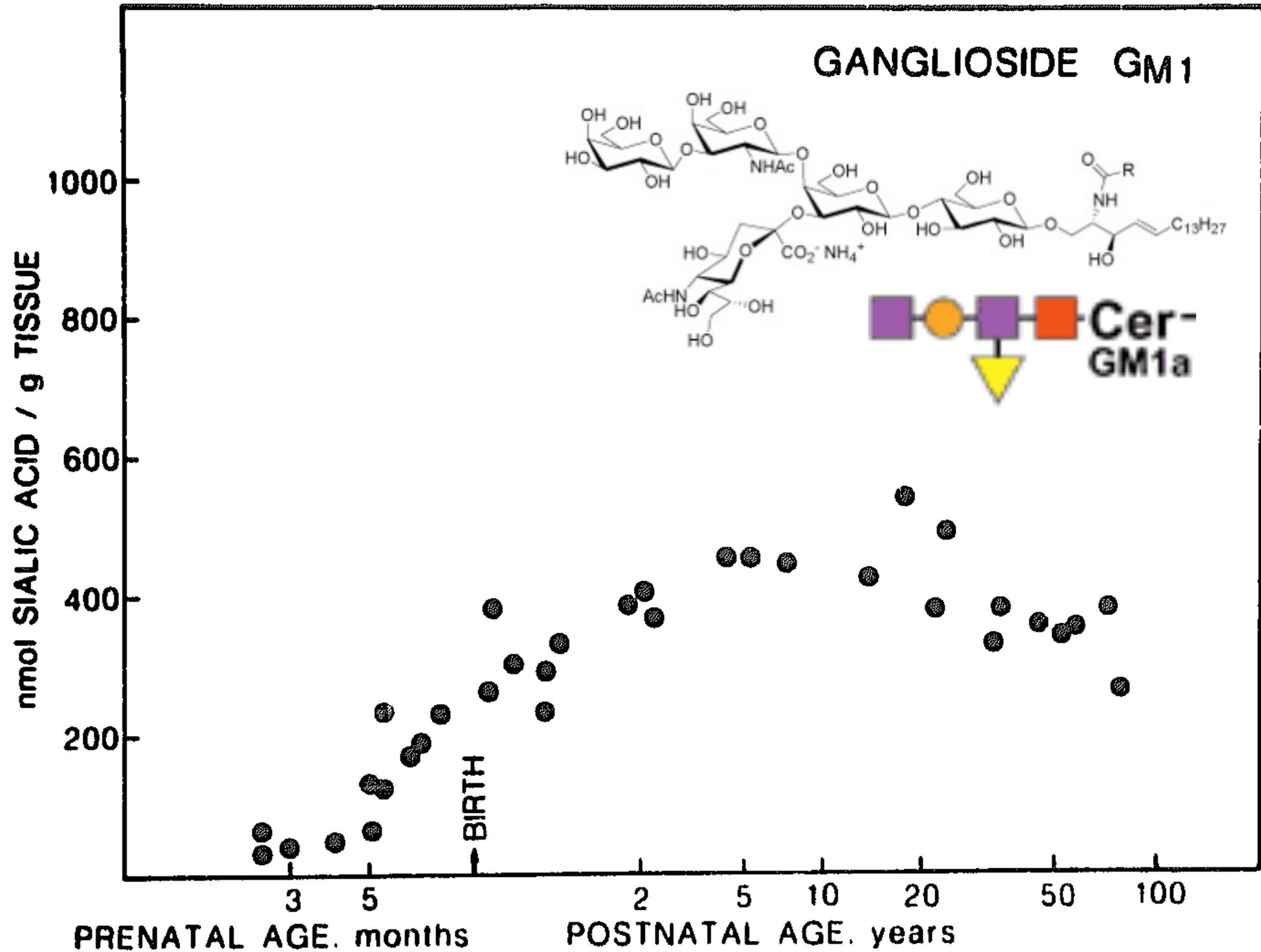


Yu R K et al. J. Lipid Res. 2009;50:S440-S445



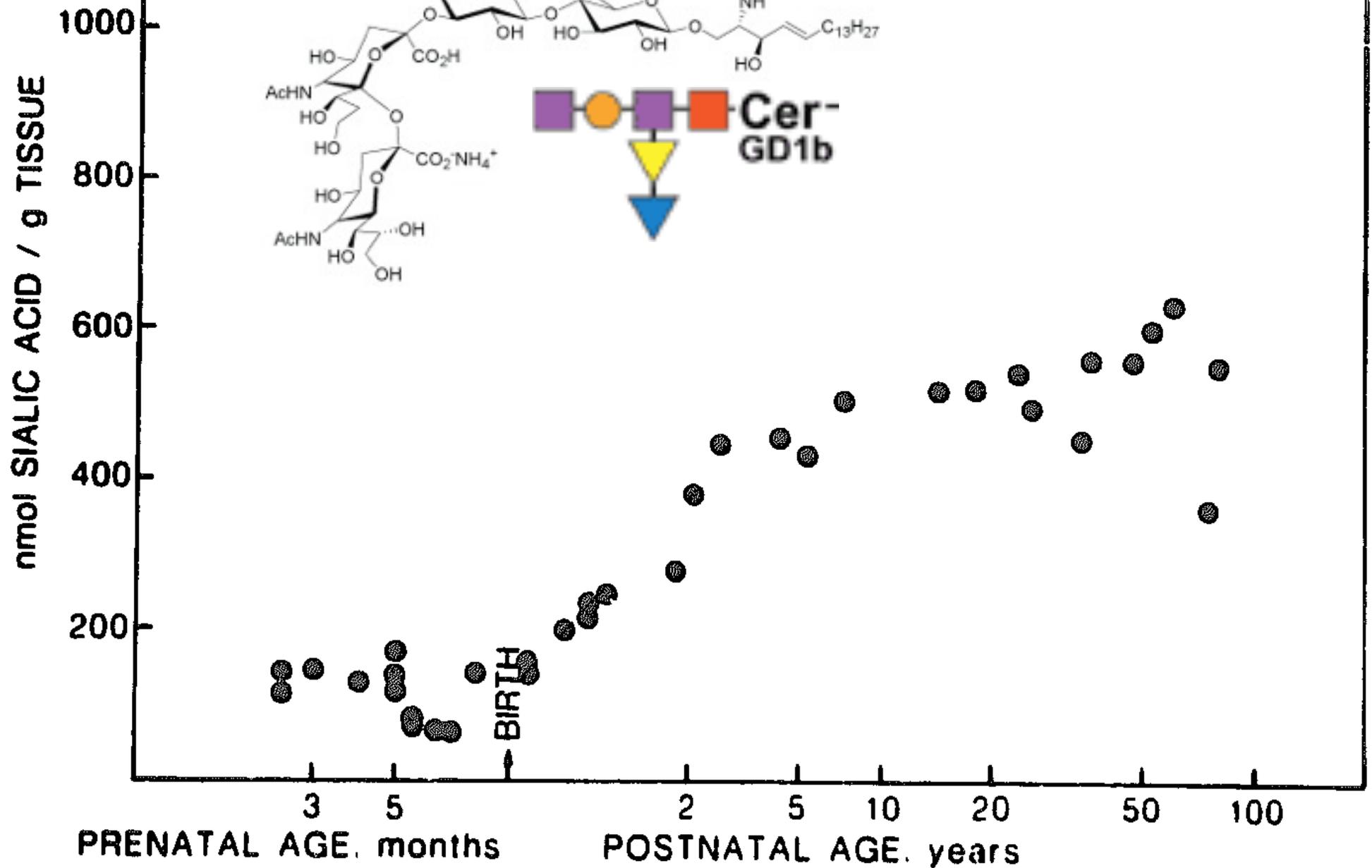


Svennerholm, L.; Boström, K.; Fredman, P.; Månsson, J. E.; Rosengren, B.; Rynmark, B. M. *Biochimica et Biophysica Acta*, **1005** (1989): 109–117.



Svennerholm, L.; Boström, K.; Fredman, P.; Månsson, J. E.; Rosengren, B.; Rynmark, B. M. *Biochimica et Biophysica Acta*, **1005** (1989): 109–117.

# GANGLIOSIDE GD1b



Svennerholm, L.; Boström, K.; Fredman, P.; Månsson, J. E.; Rosengren, B.; Rynmark, B. M. *Biochimica et Biophysica Acta*, **1005** (1989): 109–117.

# Two Goals



Obtain the  
Drug



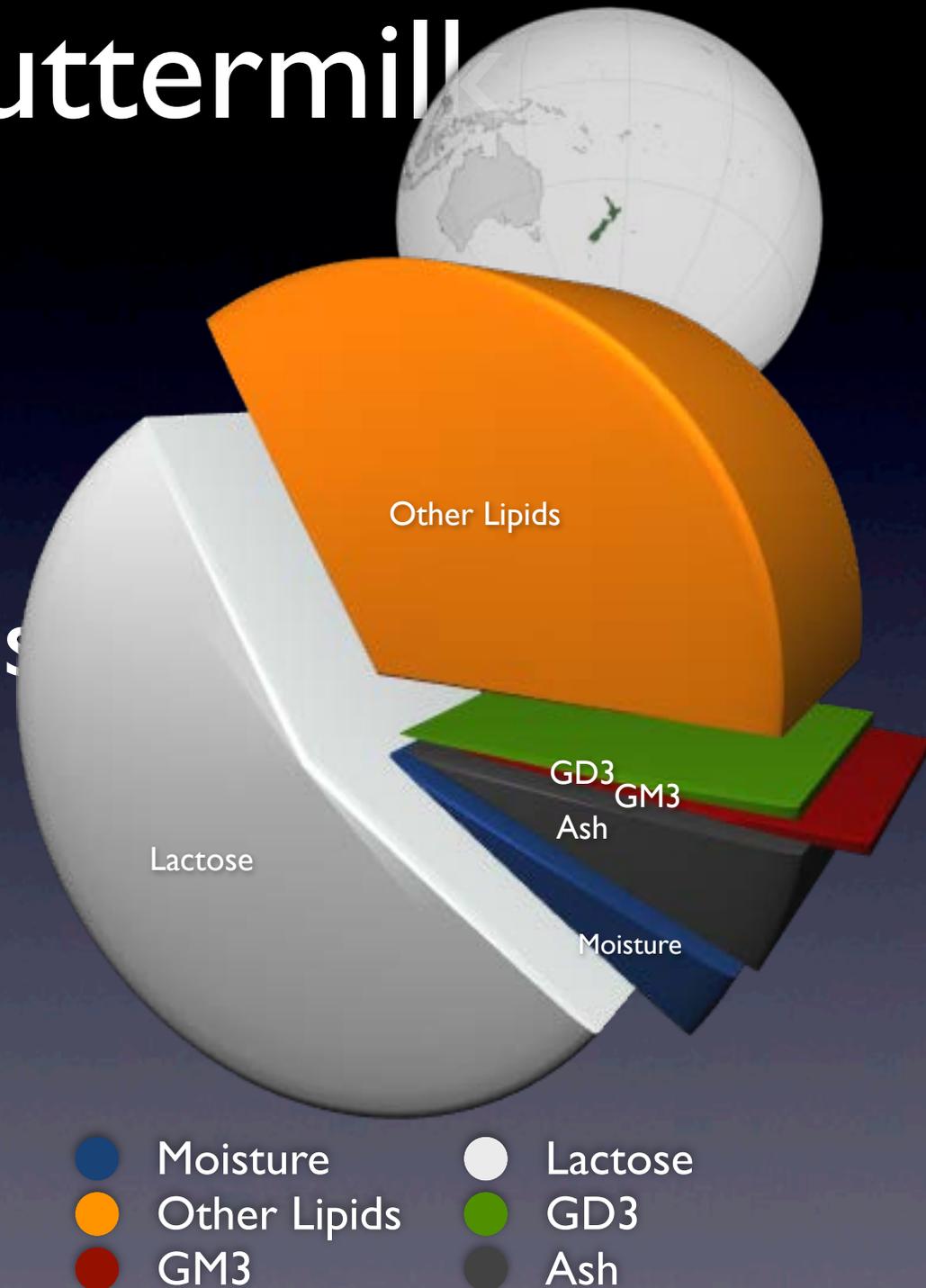
Tool to  
Monitor  
Treatment

# GM3 from Natural Sources



# anglioside 500 Buttermilk

- 0.56% GM3
- 0.6% GD3
- 34.0% other lipids
- 3.2% moisture
- 56.0% lactose
- 5.0% ash





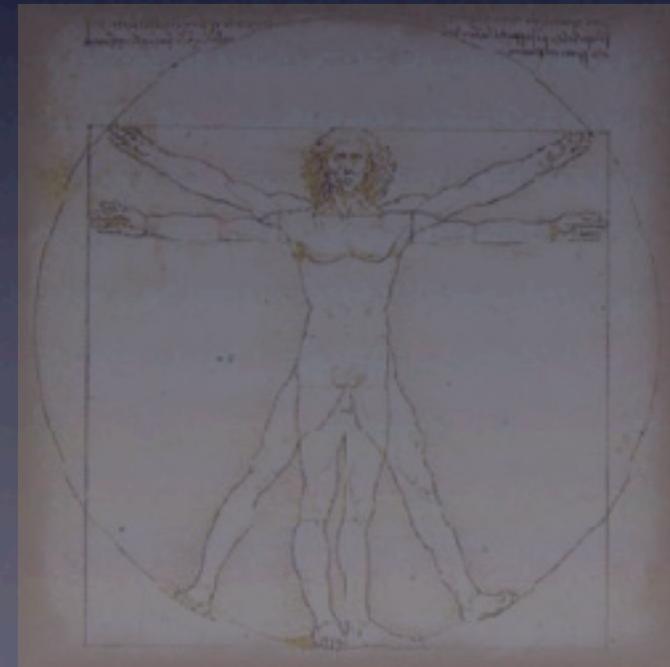
# Difficulty



GM3 from Natural Sources



≠





# Difficulty



≠



GM3 from Natural Sources



1



2

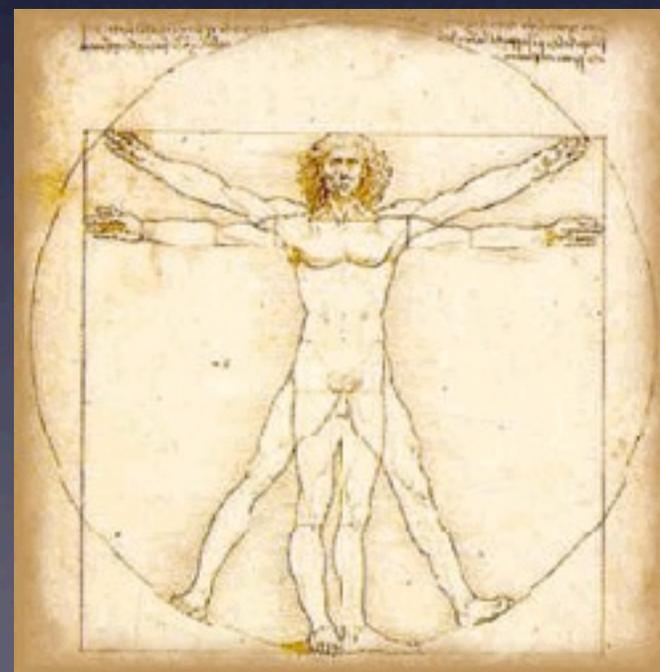
# Difficulty

3

GM3 from Natural Sources



≠



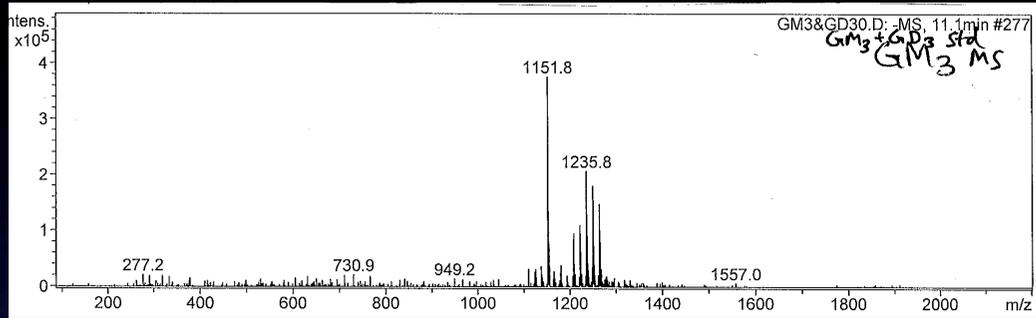
# GM3 Made in the Lab



GM3 from Natural Sources

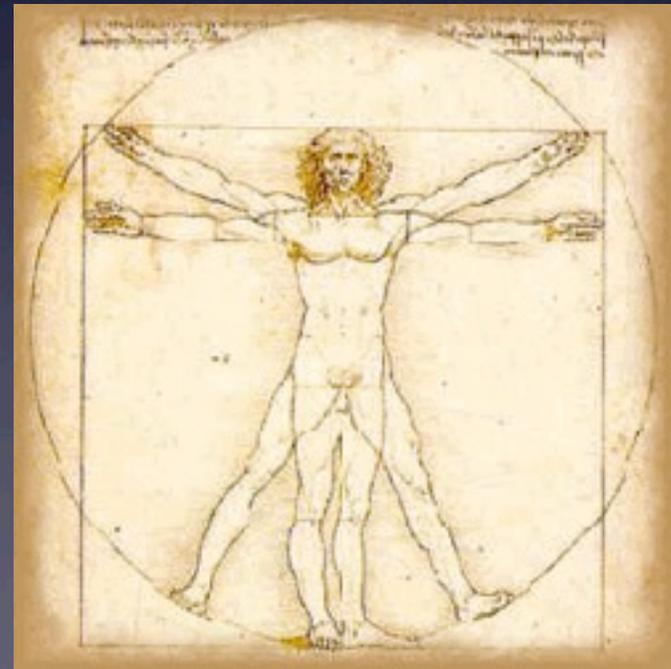
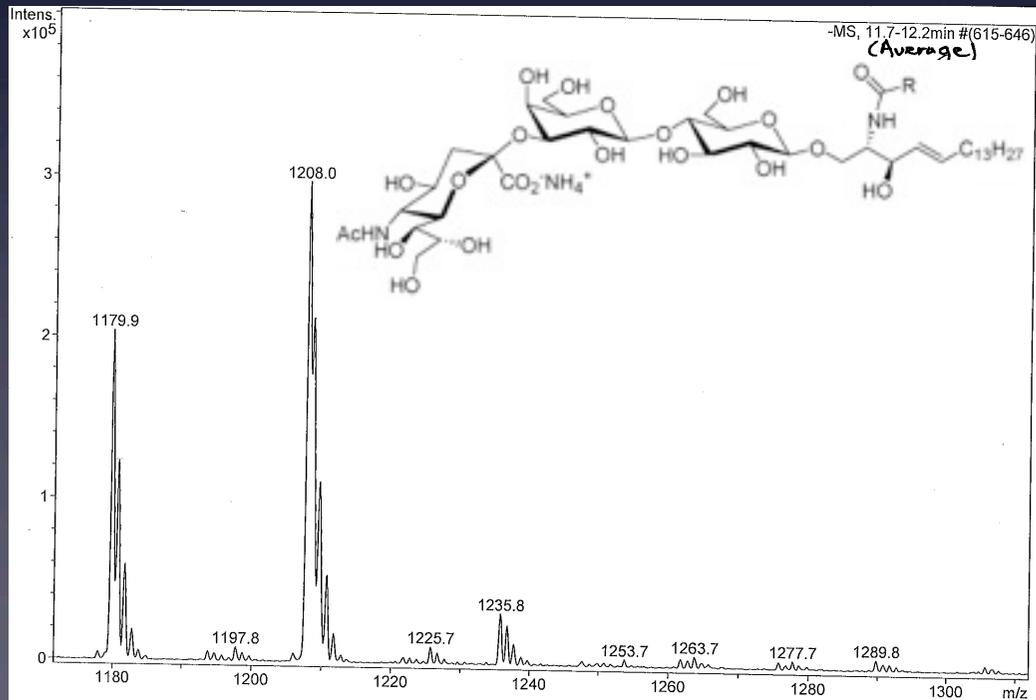


# Human vs. Cow GM3



≠

≠



# What happens after



of

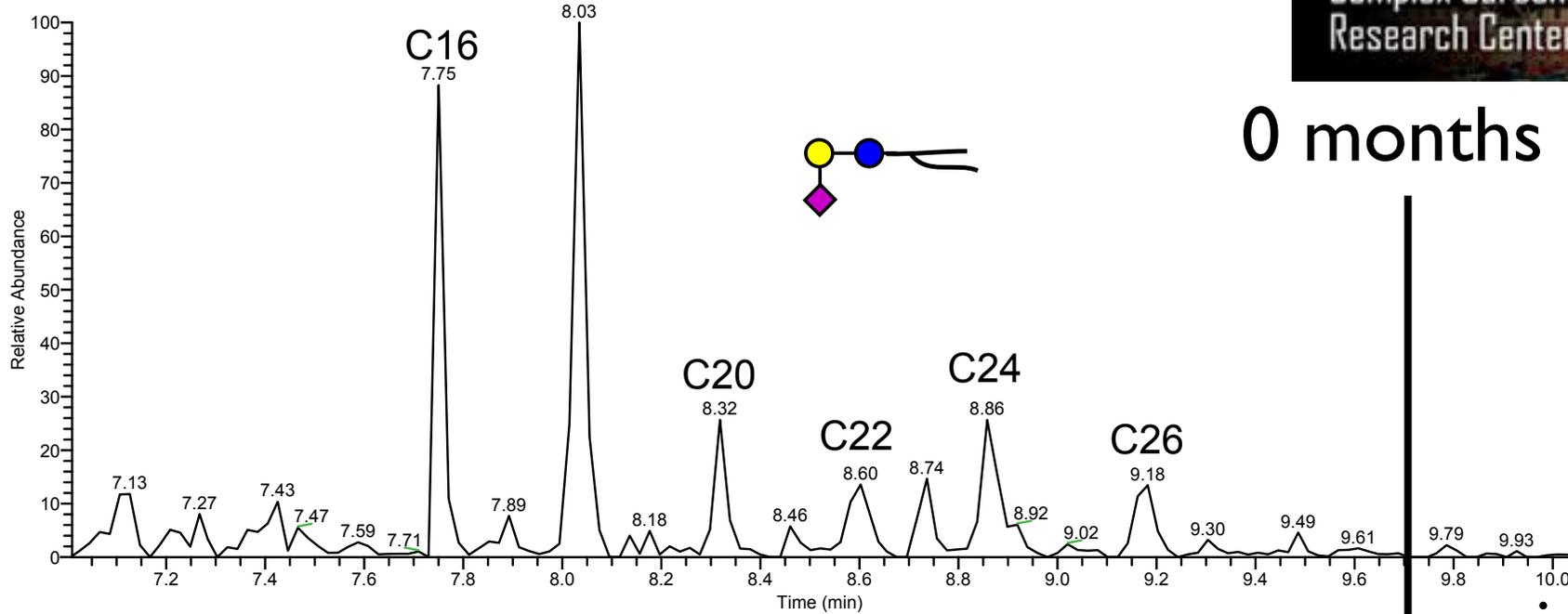


?

# Small GM3 level via placental transfer



RT: 7.01 - 10.03

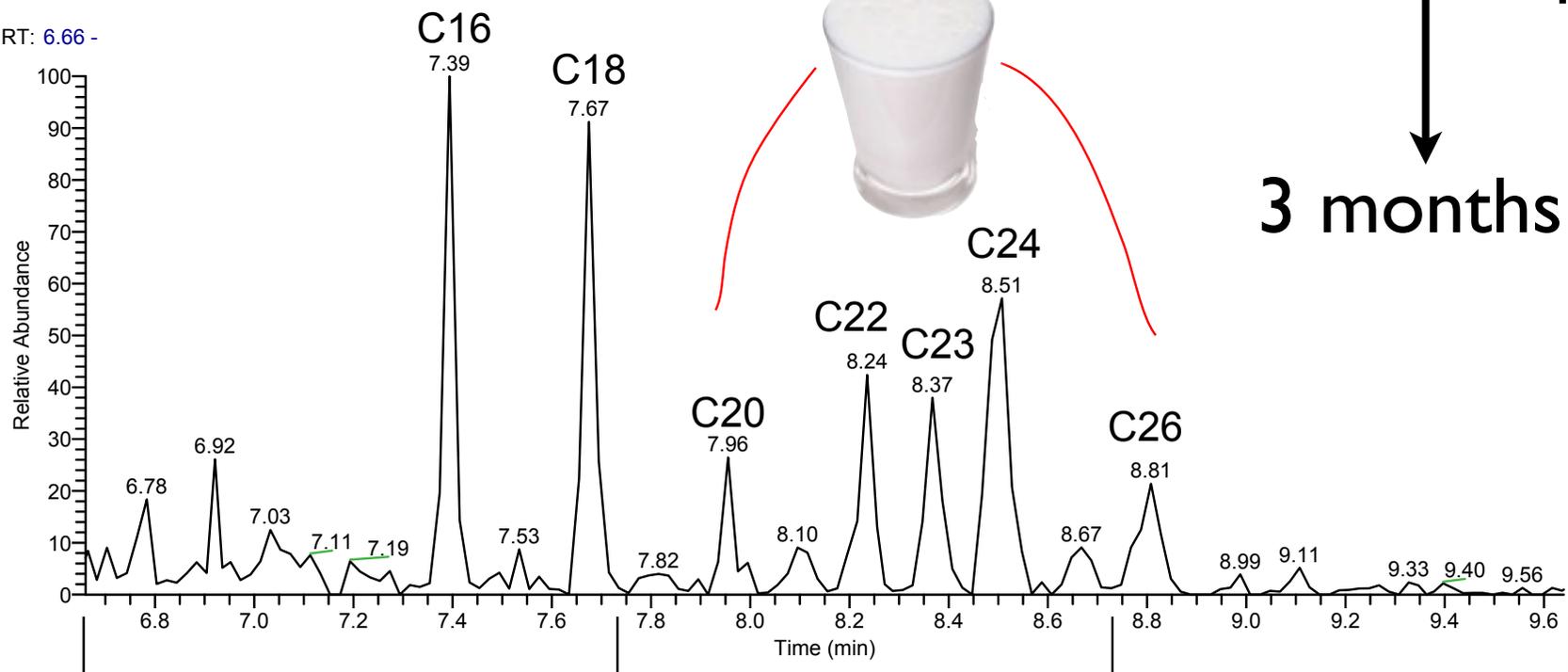


0 months

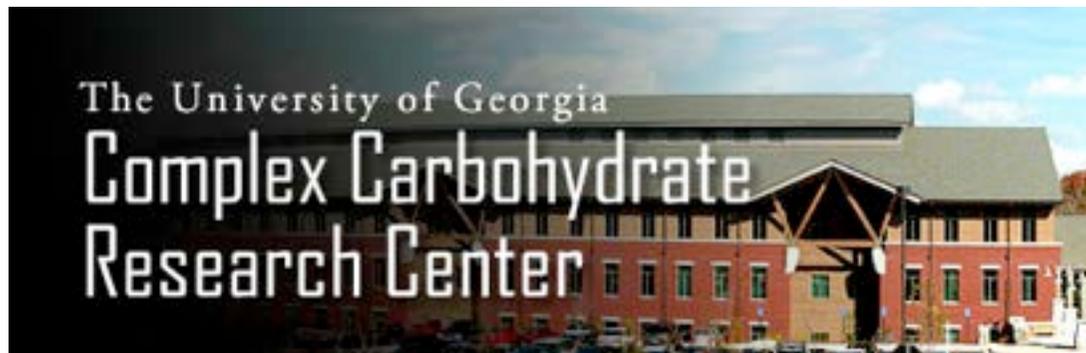
(Same affected individual)

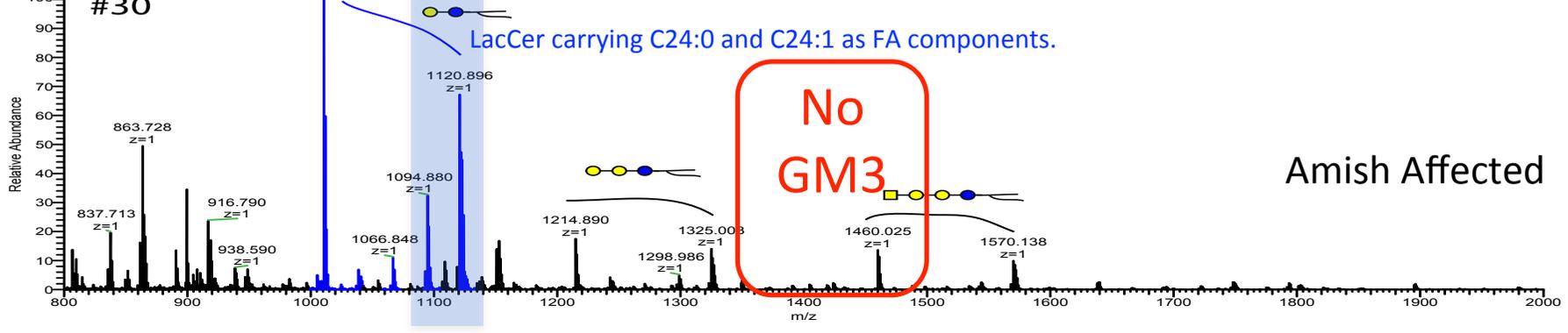
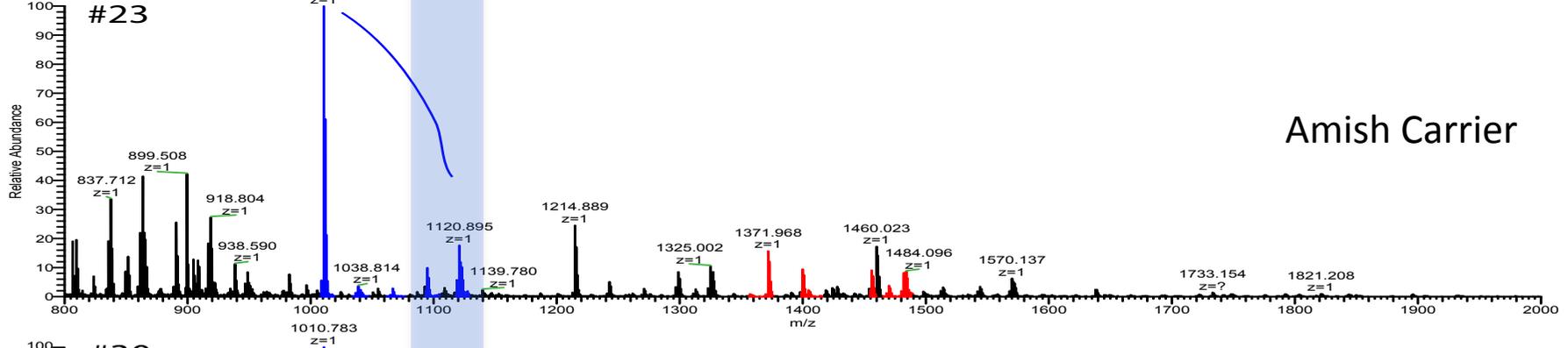
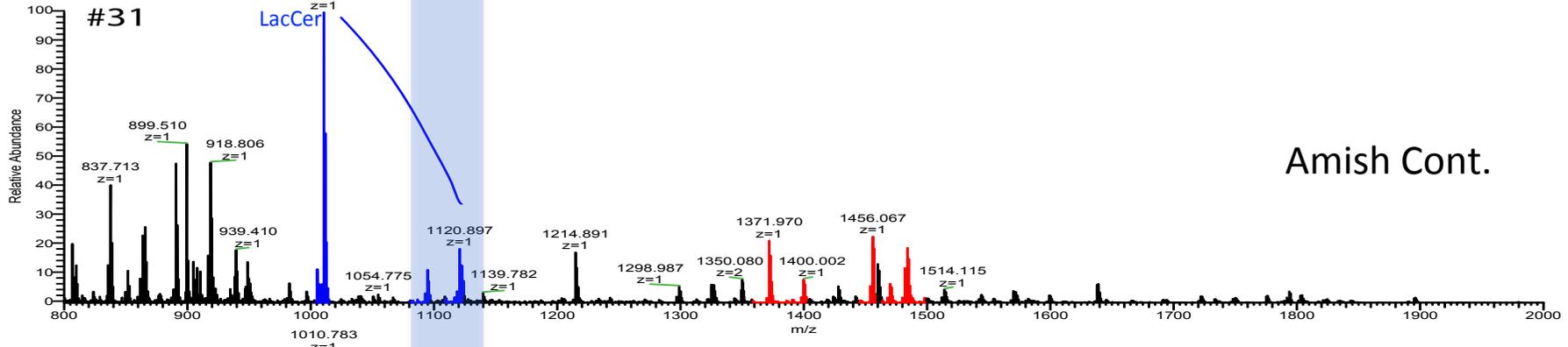
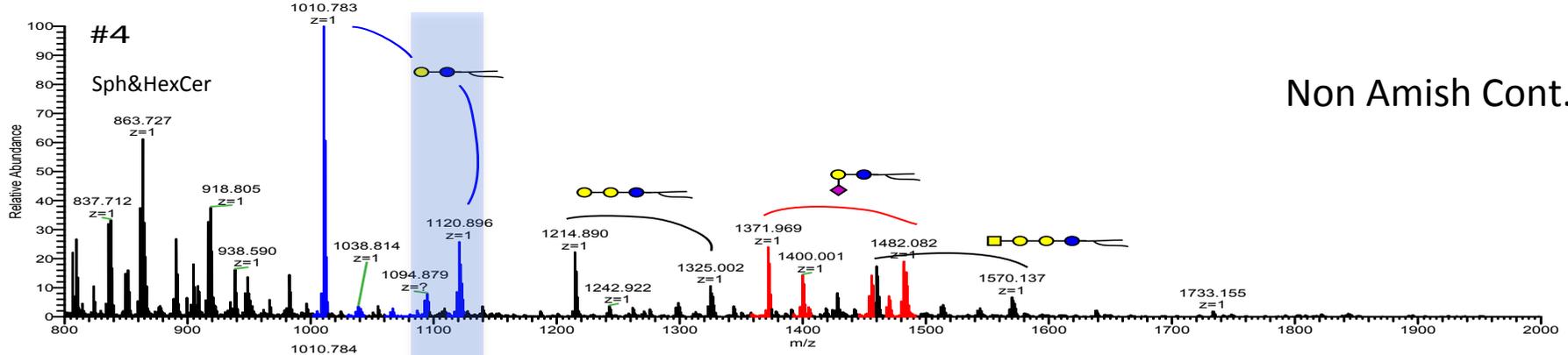
3 months

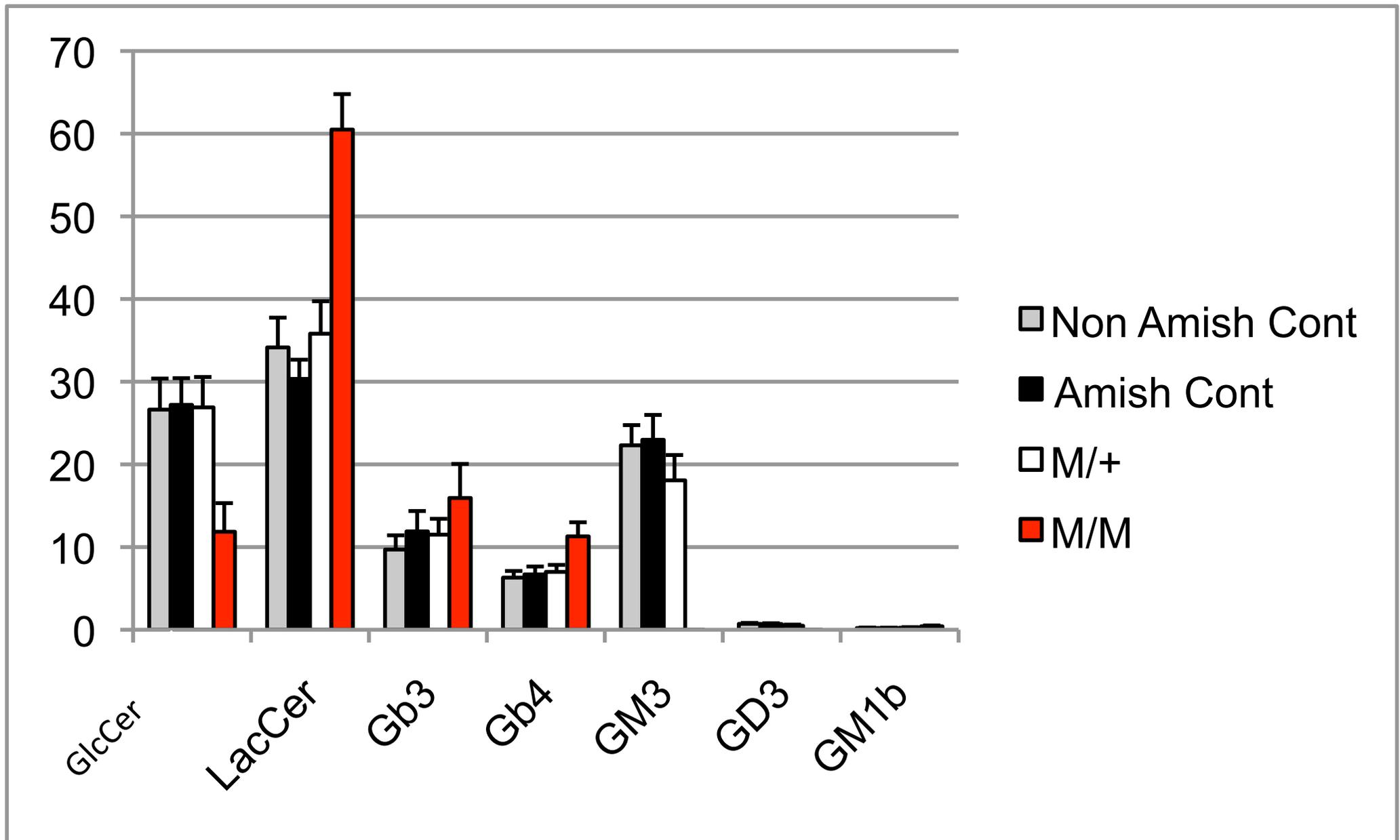
RT: 6.66 -

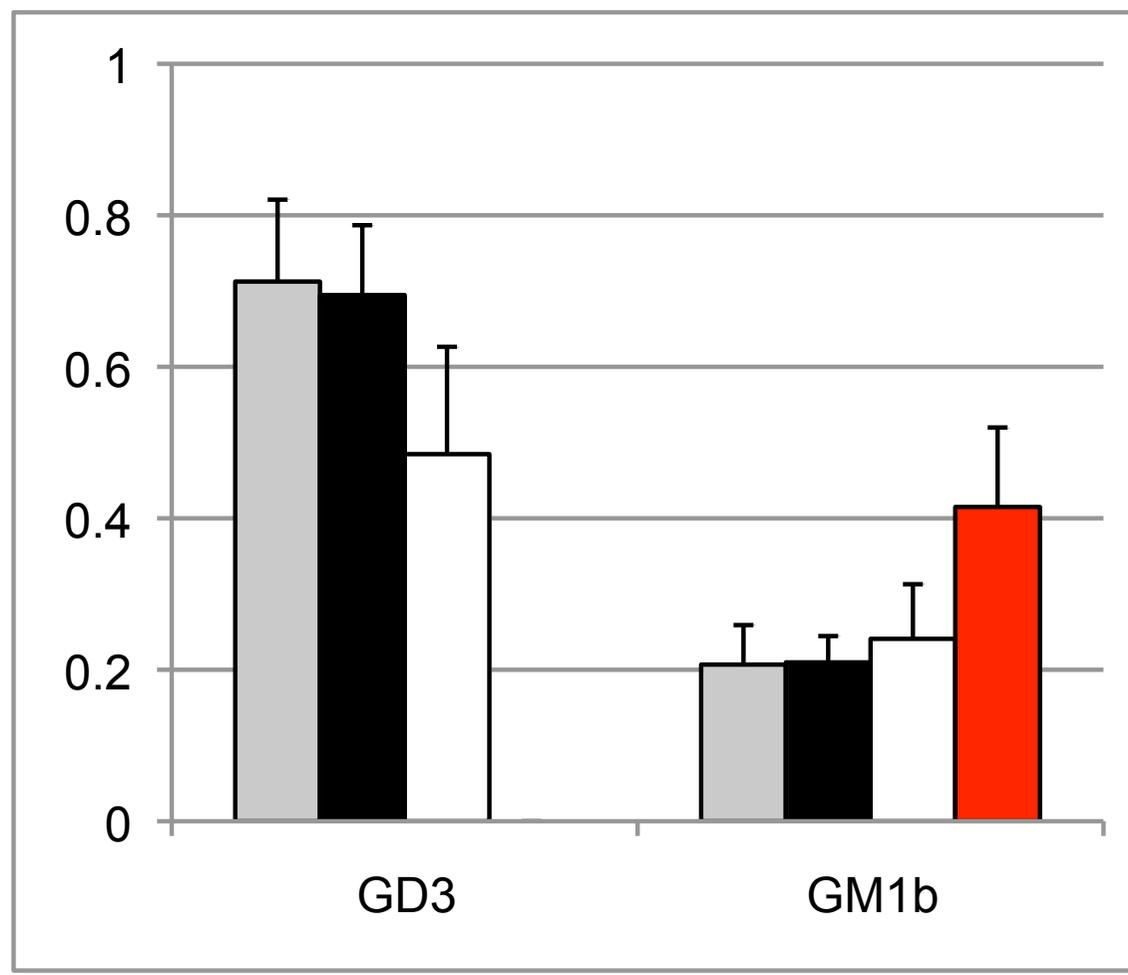
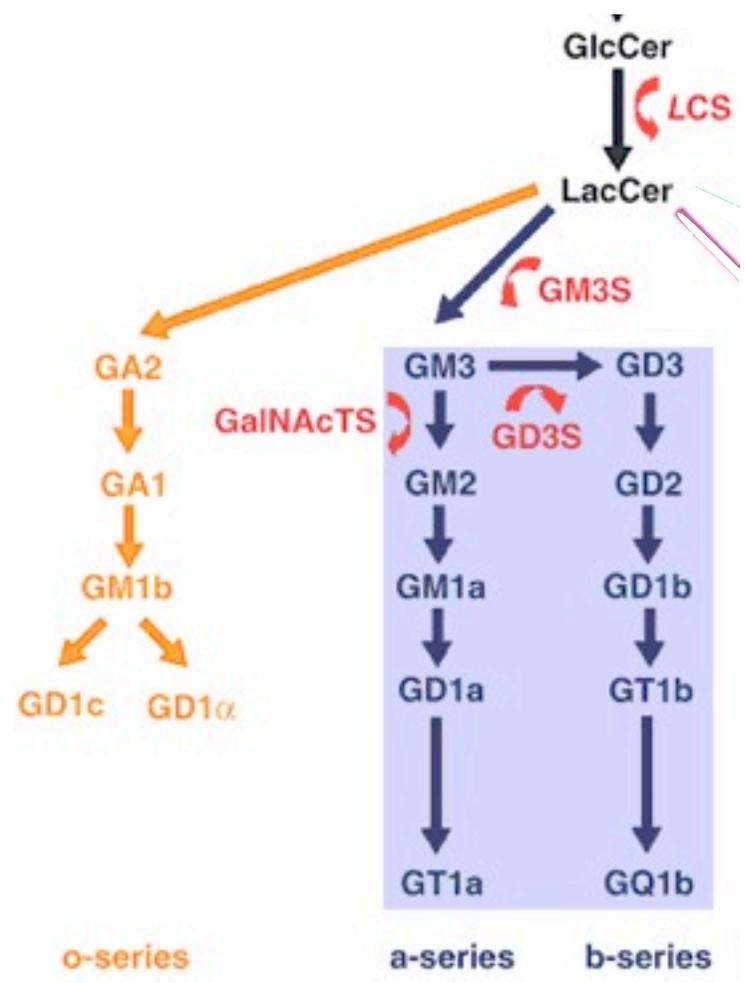
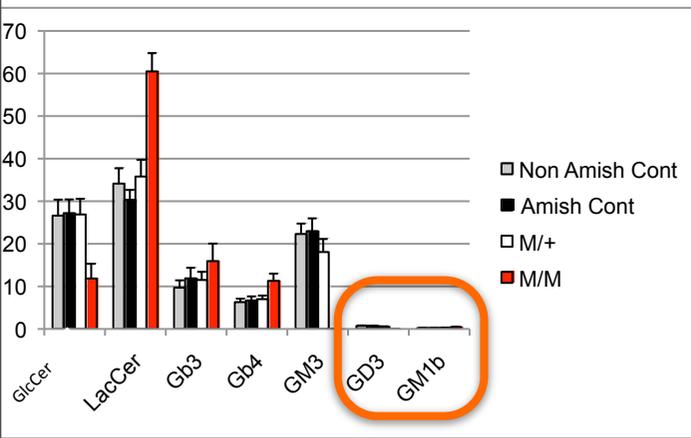


# Plasma Glycosphingolipid Levels

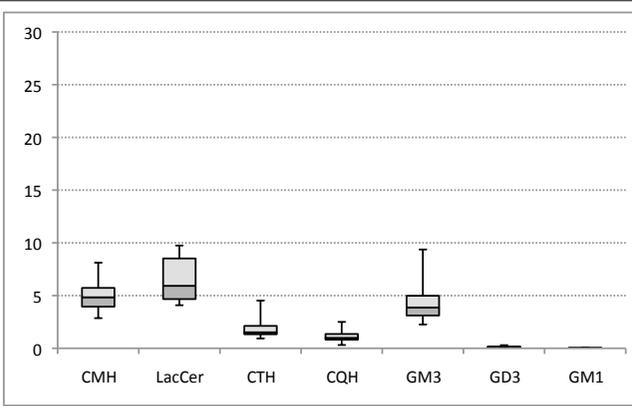




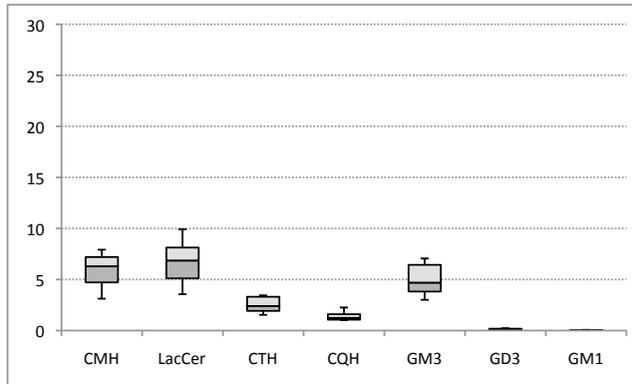




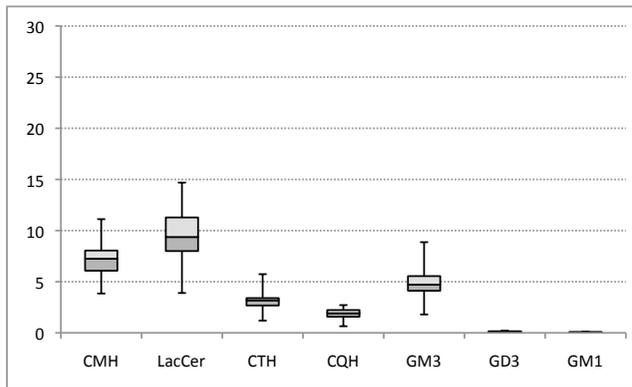
nmol/ml plasma



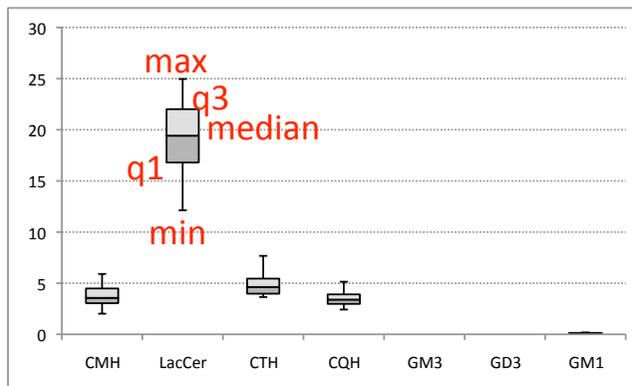
Non Amish Cont.



Amish Cont.



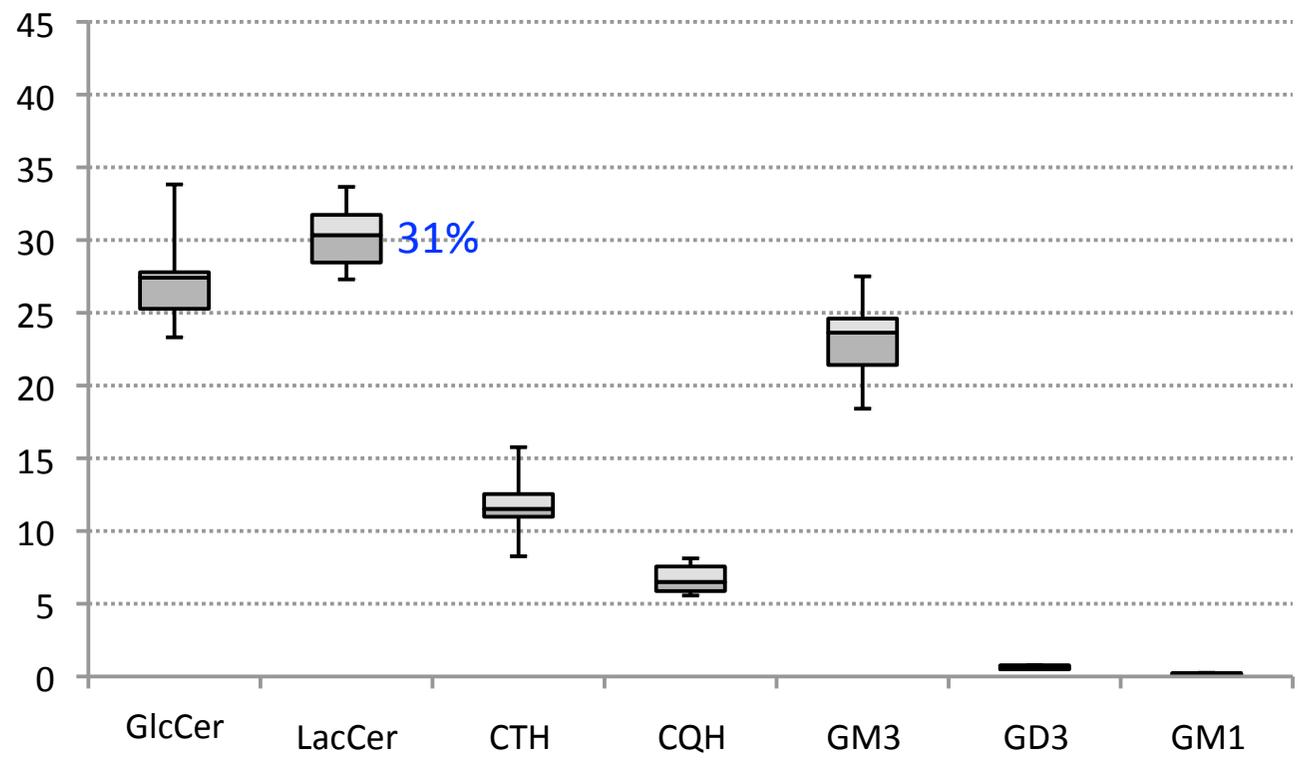
Amish Carrier



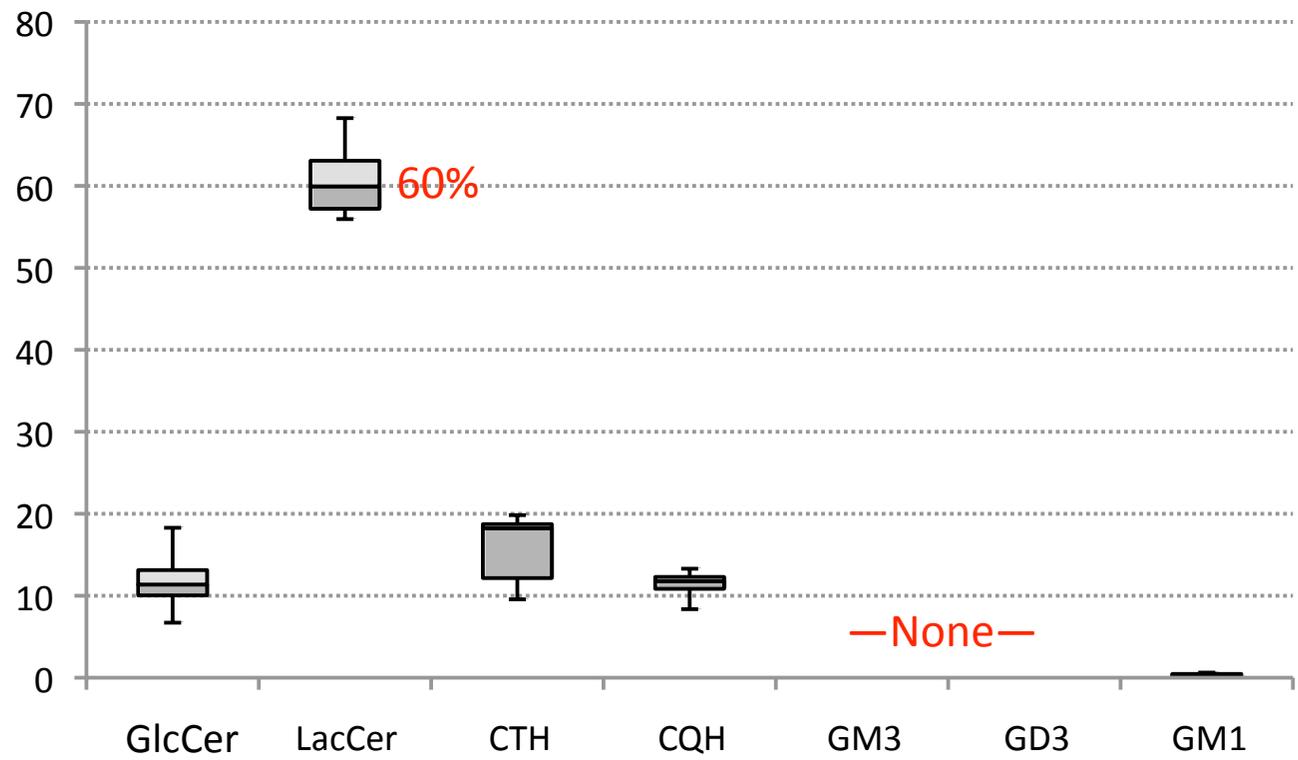
Amish Affected

Rel. %

Amish Cont.



Amish Affected





What is GM3?



**The Plan: Synthetic Strategy**



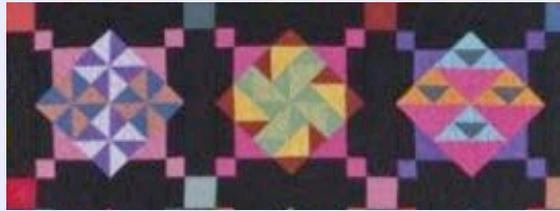
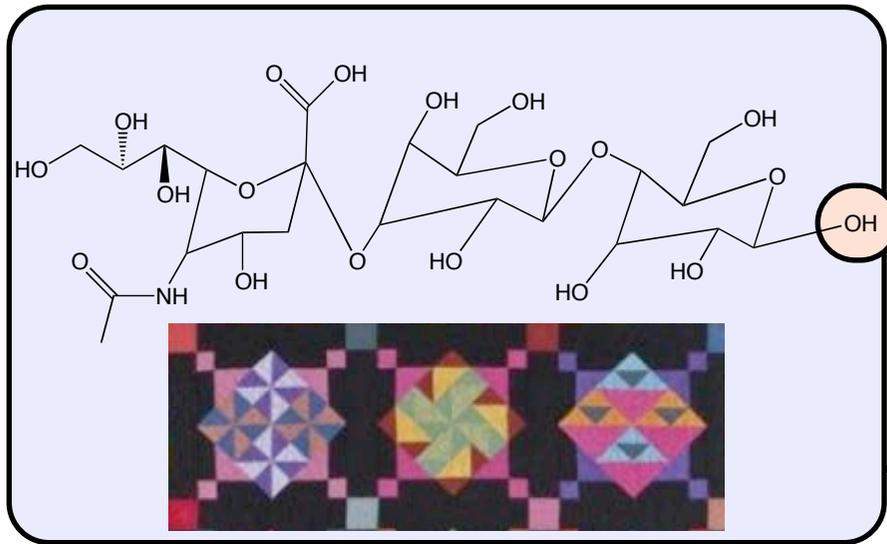
Results so far



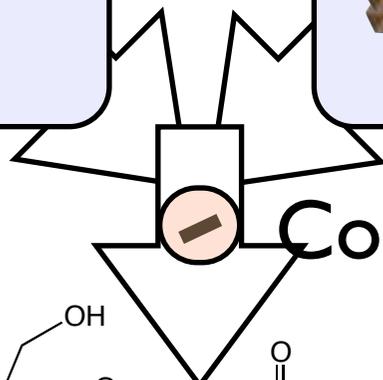
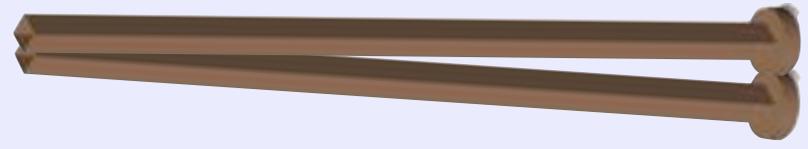
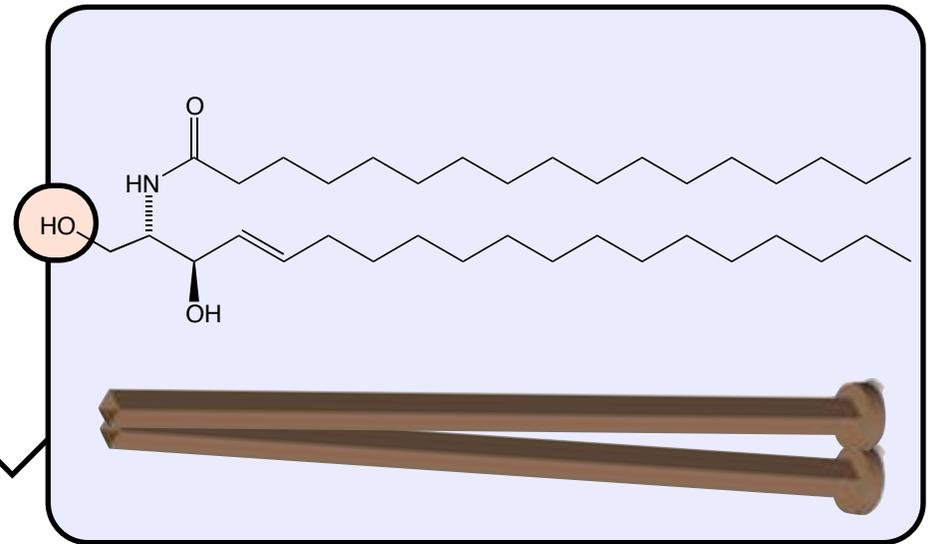
Future Directions

# Convergence

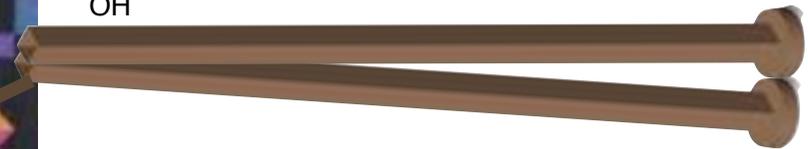
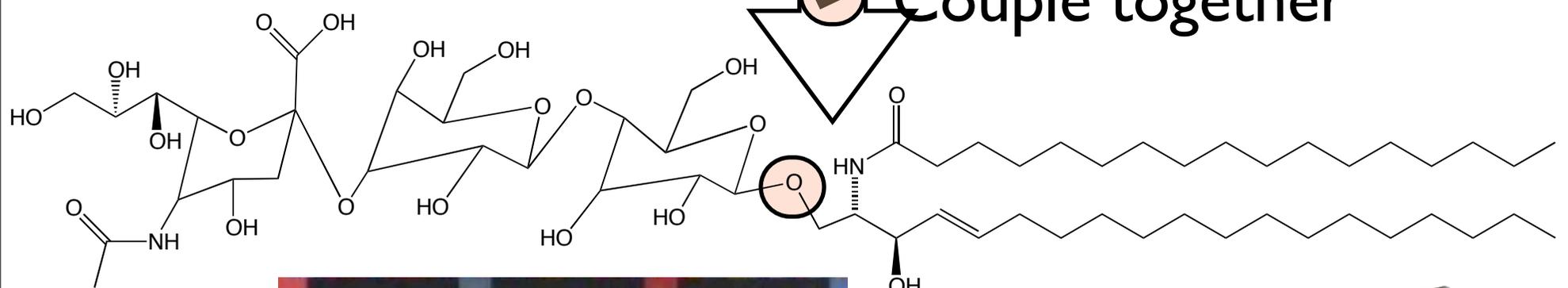
Build this up...



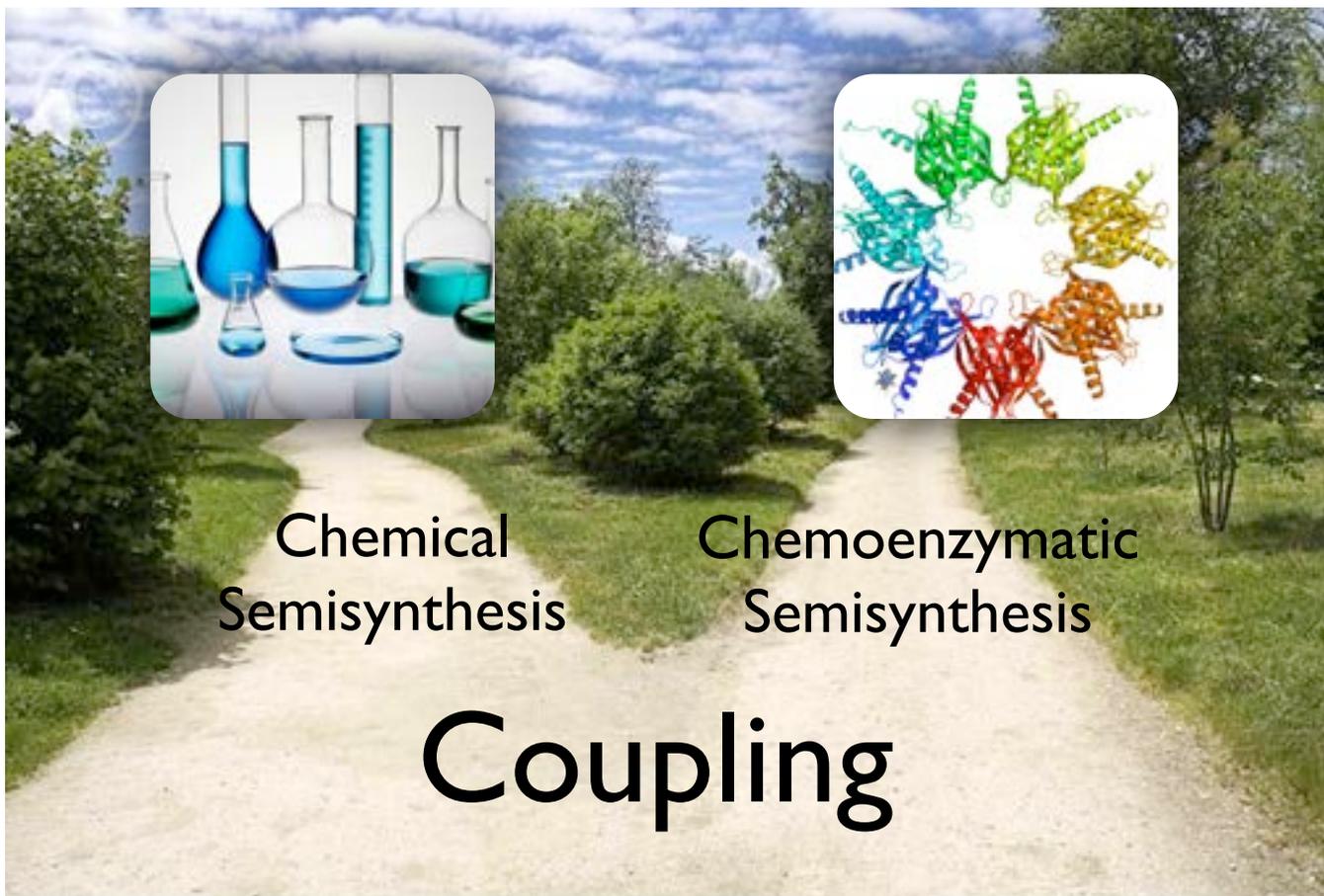
Build this up...



Couple together



# Synthetic Strategies



Chemical  
Semisynthesis

Chemoenzymatic  
Semisynthesis

Coupling

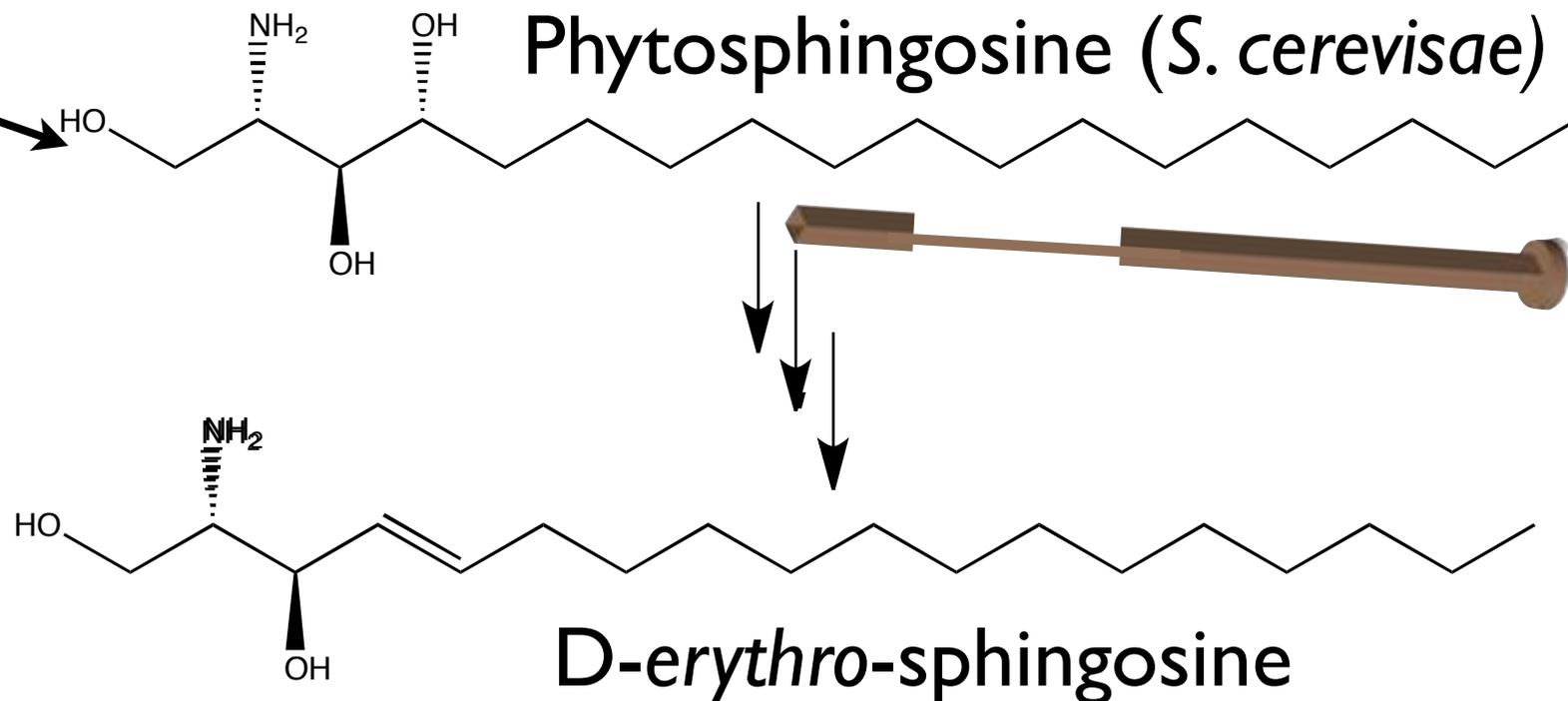
600-02461516 [RF] © www.visualphotos.com



Chemical  
Semisynthesis

## D-erythro-Sphingosine

# Preparing the pole

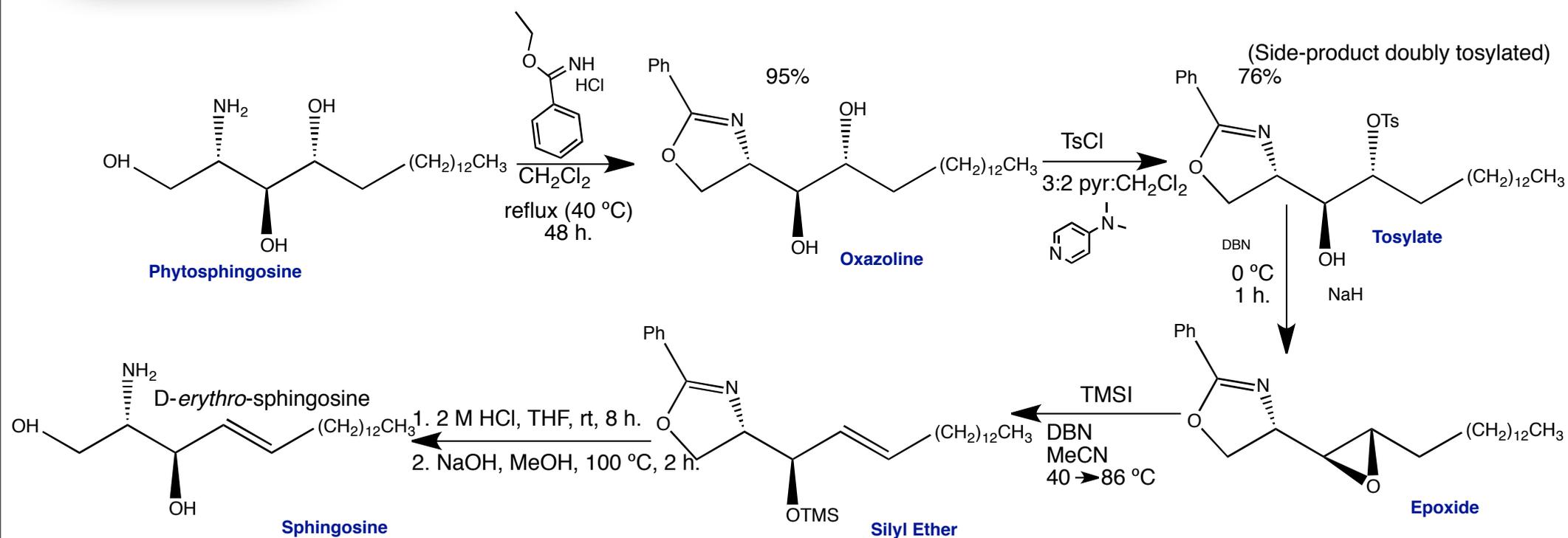


van den Berg, R. J. B. H. N. et al. *J. Org. Chem.*, **2004**, 69, 5699–5704.



## Chemical Semisynthesis

# D-erythro-Sphingosine

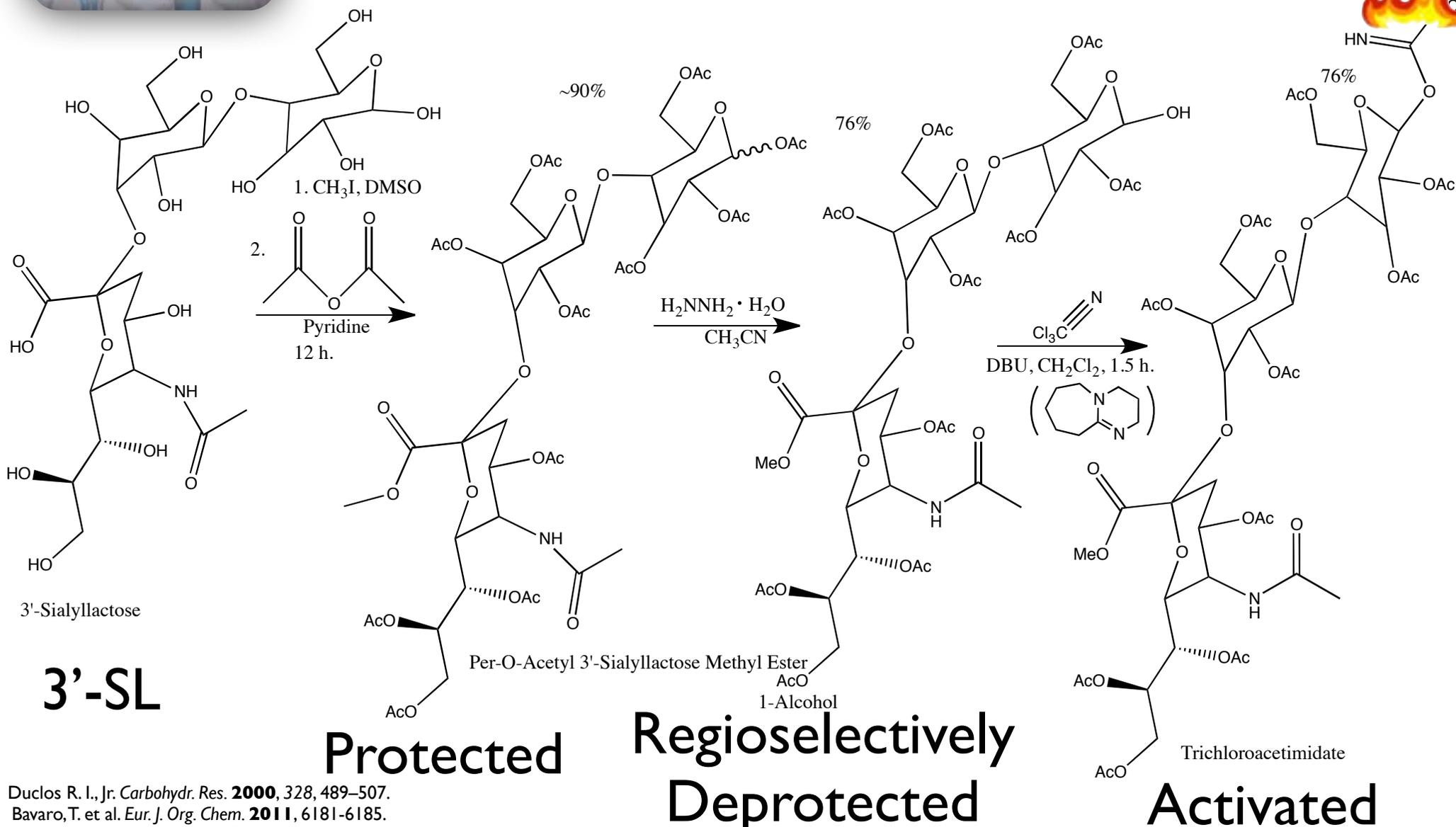


van den Berg, R. J. B. H. N. et al. *J. Org. Chem.*, **2004**, 69, 5699–5704.



# Chemical Semisynthesis

## Activating Sugar



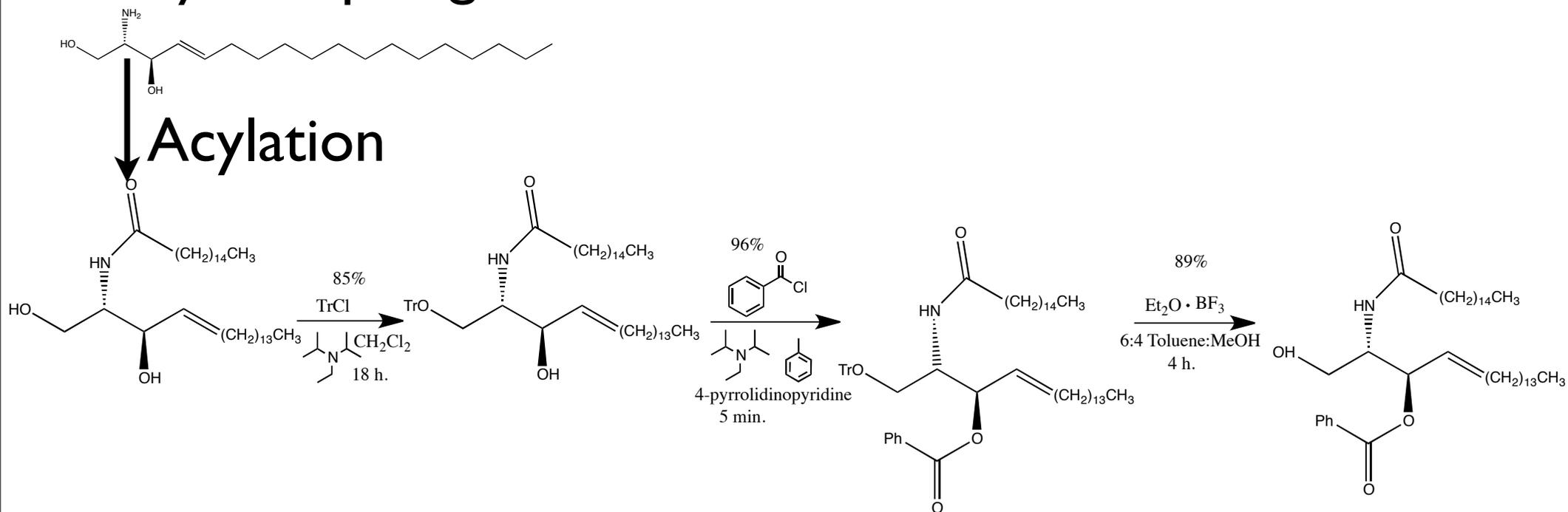
Duclos R. I., Jr. *Carbohydr. Res.* **2000**, 328, 489–507.  
Bavaro, T. et al. *Eur. J. Org. Chem.* **2011**, 6181–6185.  
Khan, R. et al. *Aust. J. Chem.* **1996**, 49, 293–298.



# Chemical Semisynthesis

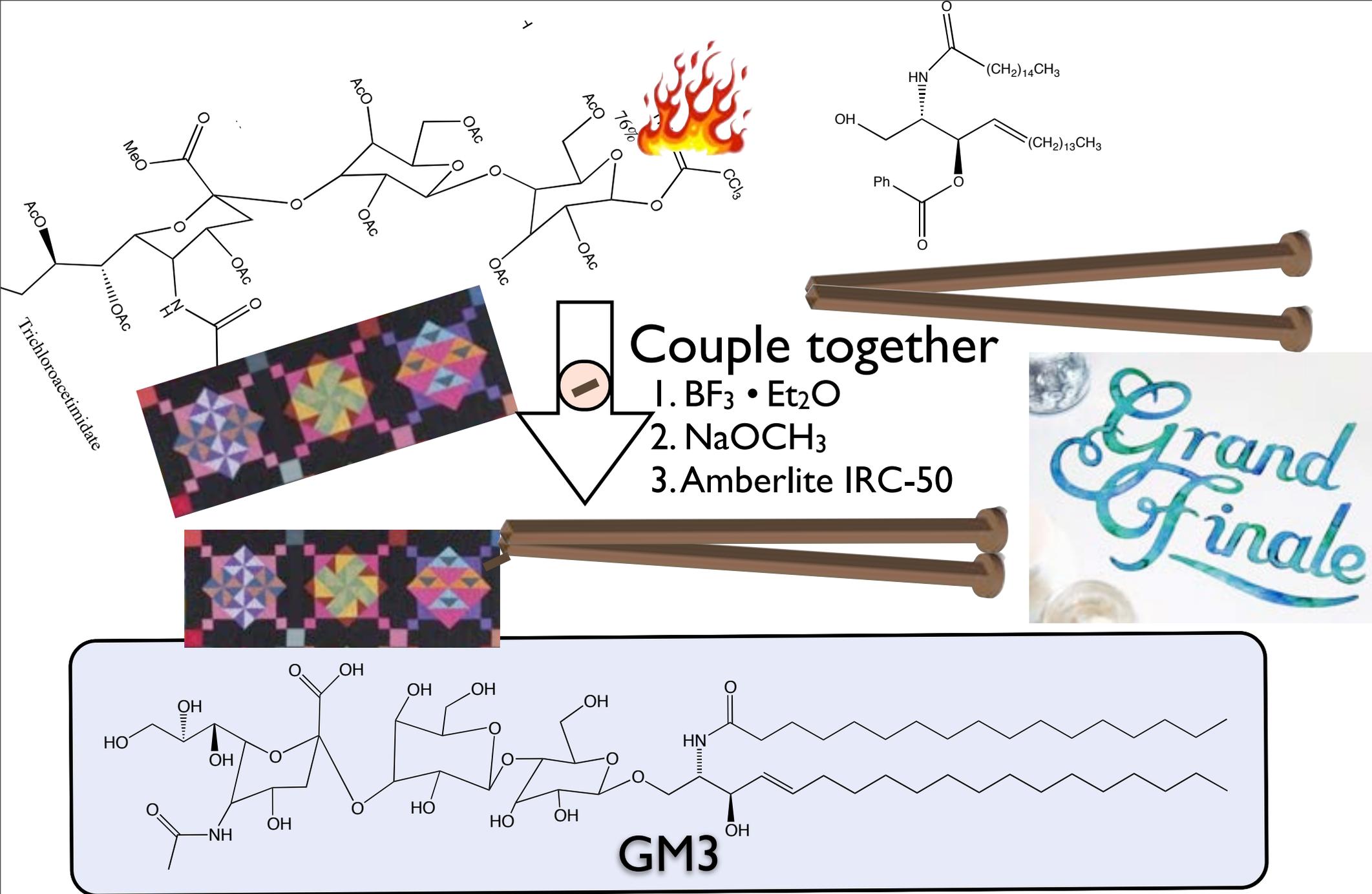
## Ceramide Preparation

### D-erythro-sphingosine



Duclos R. I., Jr. *Carbohydr. Res.* **2000**, 328, 489–507.

Rai, A. N.; Basu, A. *Org. Lett.* **2004**, 6, 2861–2863.



Duclos R. I., Jr. *Carbohydr. Res.* **2000**, 328, 489–507.



What is GM3?



Synthetic Strategy



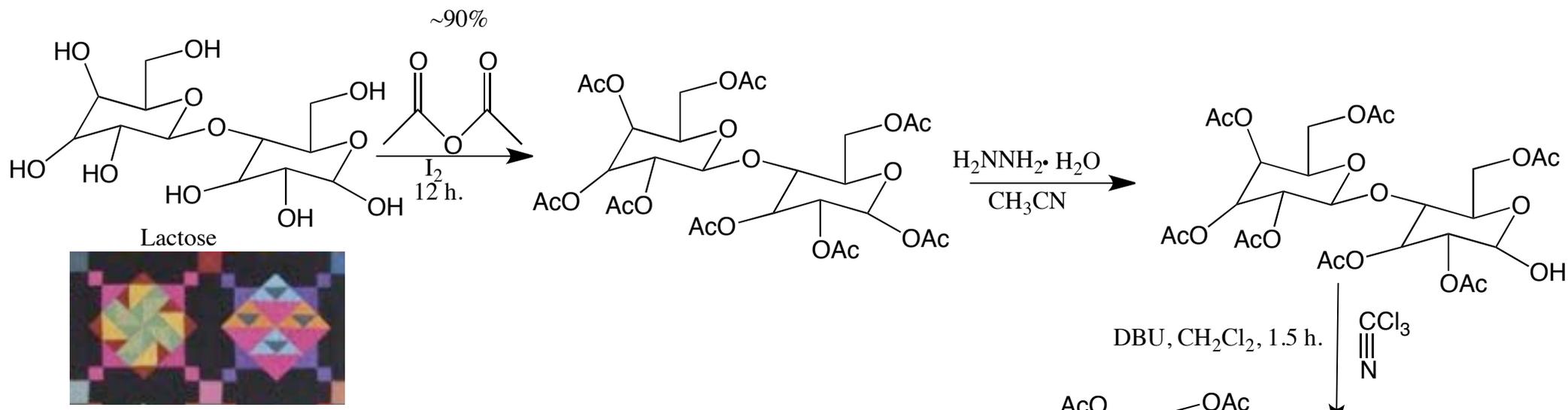
**Results so far**



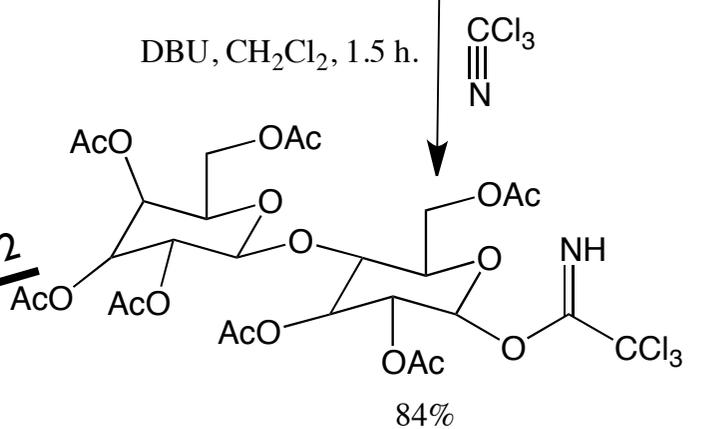
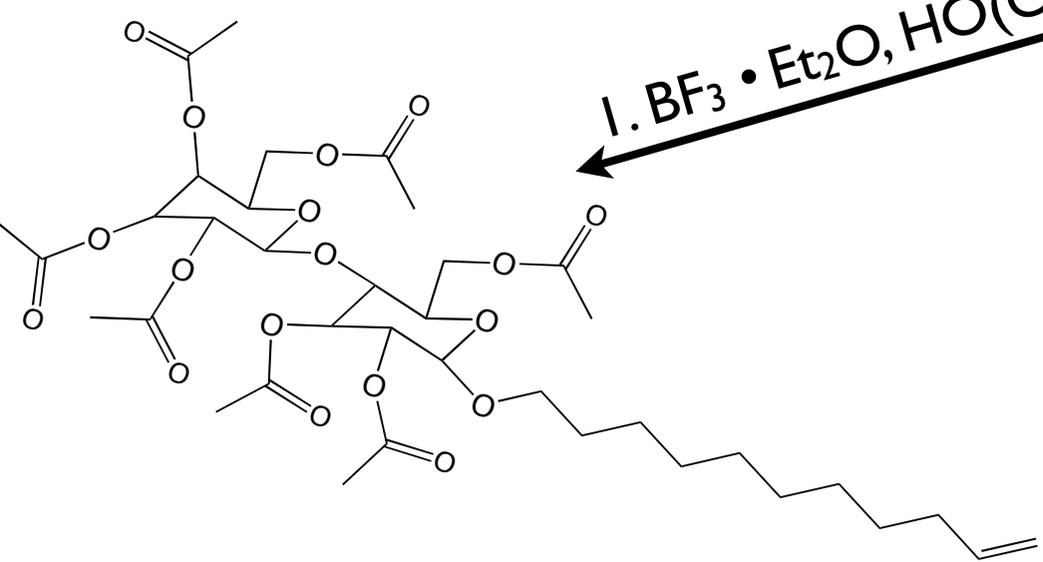
Future Directions



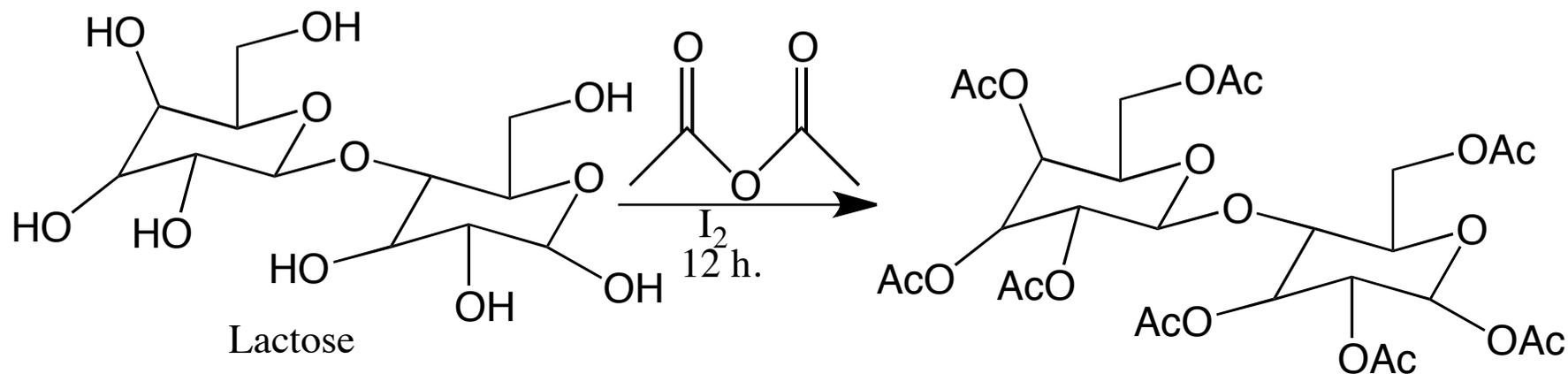
# Model Coupling



$\xrightarrow{\text{I. BF}_3 \cdot \text{Et}_2\text{O}, \text{HO}(\text{CH}_2)_9\text{CH}=\text{CH}_2}$



# 8-O-acetyl lactose

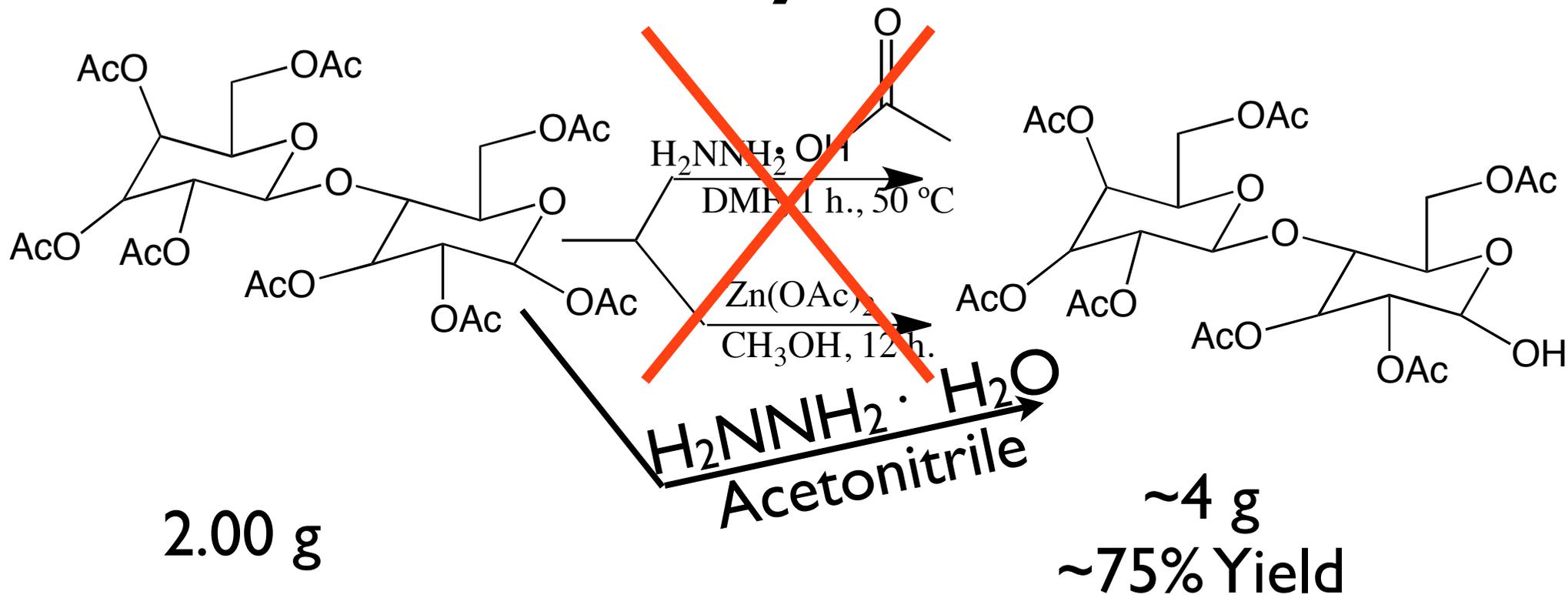


5.63 g

10.67 g

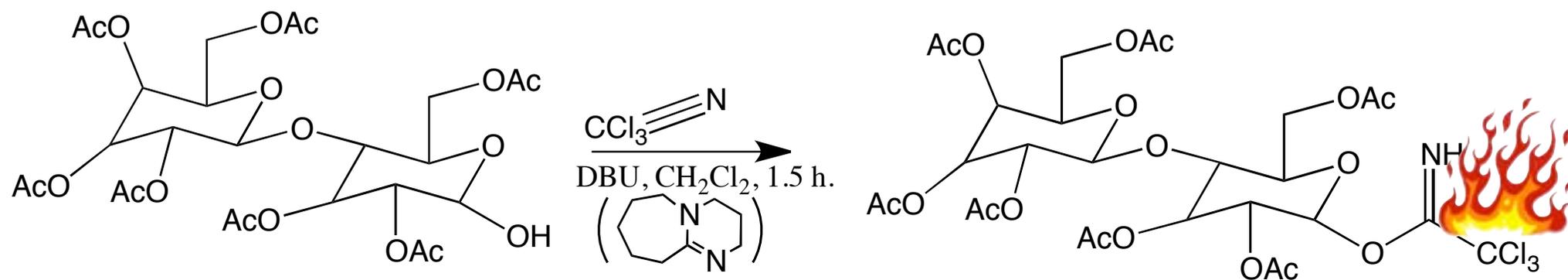
95.52% Yield

# 7-O-acetyl lactose



Duclos R. I., Jr. *Carbohydr. Res.* **2000**, 328, 489–507.  
Kaya, E.; Sonmez, F.; Kucukislamoglu, M.; Nebioglu, M. *Chem. Pap.* **2012**, 66, 312–315.  
Khan, R. et al. *Aust. J. Chem.* **1996**, 49, 293–298.

# 7OAc Lac I-Trichloroacetimidate

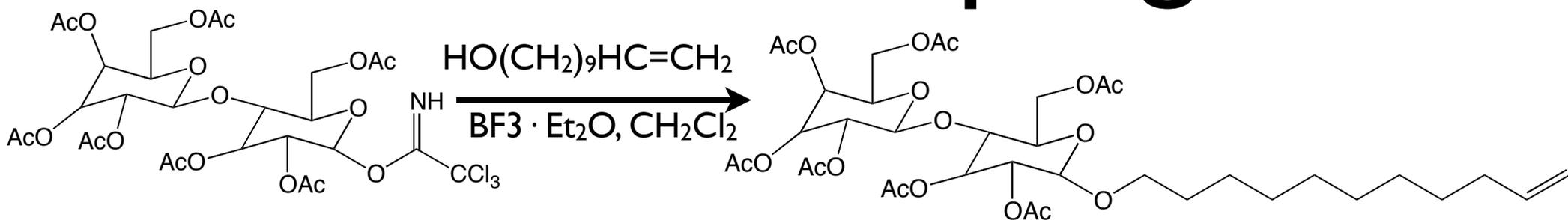


Performed on 400 mg scale

Tried NaH instead of DBU (100 mg scale)

Duclos R. I., Jr. *Carbohydr. Res.* **2000**, 328, 489–507.  
Schmidt, R. R.; Michel, J. *Angew. Chem. Int. Ed. Engl.* **1980**, 19, 731–732.

# Model Coupling



Performed on 300 mg scale

One-pot event after trichloroacetimidate formation

MS provides evidence of successful coupling



What is GM3?



Synthetic Strategy



Results so far



Future Directions

# Finish Synthesis!

The image features a map of the United States where the landmass is filled with numerous stacks of US dollar bills, bound with blue rubber bands. The stacks are arranged in a way that they appear to be piled up across the entire country. In the center of the map, there is a rectangular inset containing a colorful quilted pattern, specifically a row of three distinct quilt blocks: a purple and white star, a yellow and green floral, and a pink and blue geometric design. The background of the map is a dark, gradient blue.

Make the Sugar Here!

depositphotos depositphotos depositphotos

# Engineering a cell line to produce 3'-sialyllactose

Journal of Biotechnology 134 (2008) 261–265



Contents lists available at [ScienceDirect](#)

Journal of Biotechnology

journal homepage: [www.elsevier.com/locate/jbiotec](http://www.elsevier.com/locate/jbiotec)



## Genetic engineering of *Escherichia coli* for the economical production of sialylated oligosaccharides

Nicolas Fierfort, Eric Samain\*

*Centre de Recherches sur les Macromolécules Végétales (CERMAV – CNRS), affiliated with Joseph Fourier University and member of the ICMG (Institut de Chimie Moléculaire de Grenoble), BP 53, 38041 Grenoble Cedex 9, France*



1. Establish Safety

2. Proof of Principal

Make ~1–2 g

Use mouse model



Clinical Trial



Tuesday, July 23, 13

# Acknowledgements

## Funding

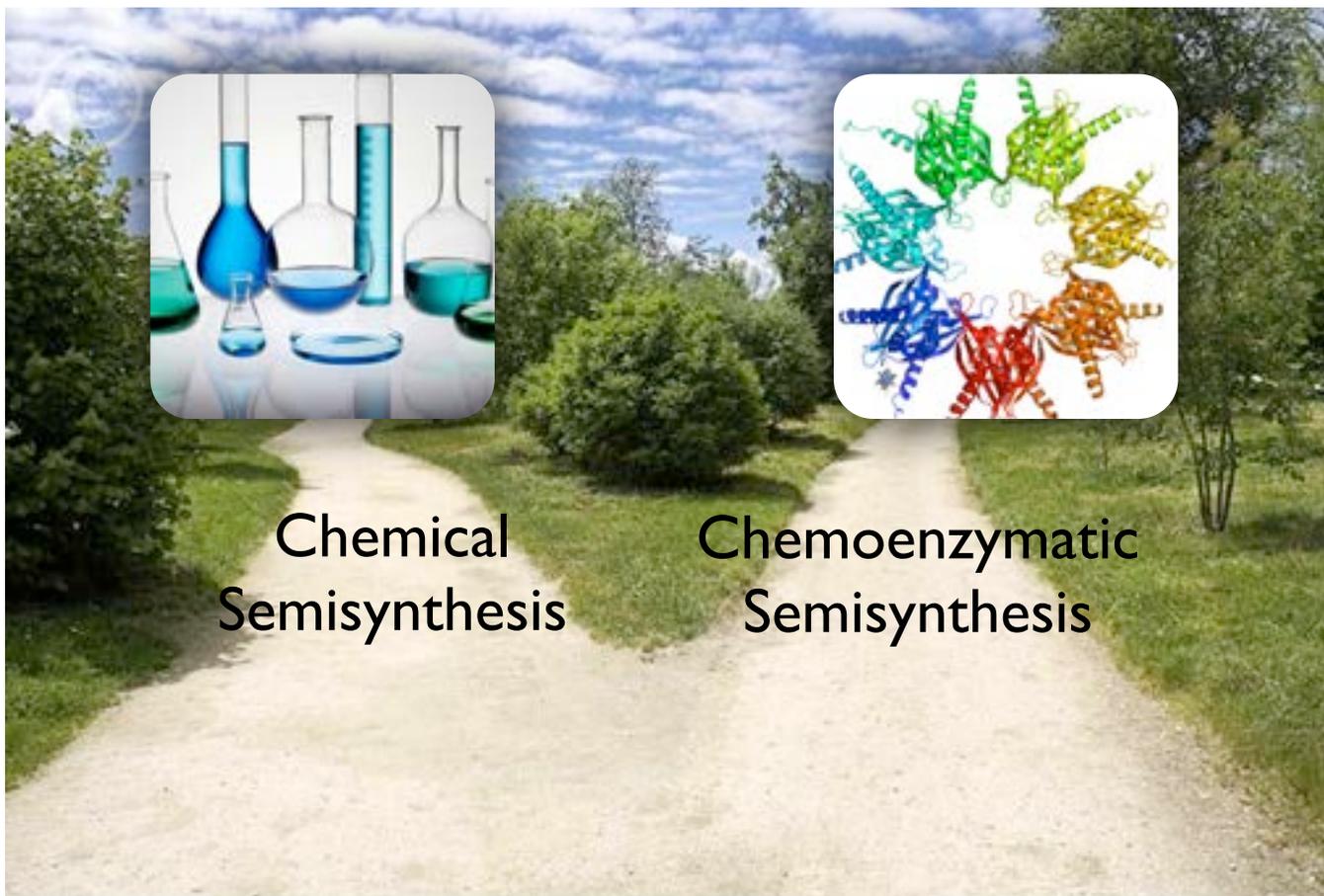
- Dr. Hess
  - Dr. Van Arman
  - Dr. Brewer
  - Dr. Fenlon
  - Dr. Piro
- F&M Chemistry Department
  - Hackman Scholars Fund
  - Dr. Eric Rackow
  - HHMI
  - Eyer Grant

## Collaborators

- Dr. Rob Jinks
  - Dr. Kevin Strauss
  - Dr. D. Holmes Morton
  - Dr. Stephen Roth
  - Dr. Matthew Kremer
  - Dr. Theresa Swenson
  - Adam Heaps
  - Dr. Stephen G. Withers
  - Dr. Shawn DeFrees
- Jon Salandra

Special Thanks to Lisa Mertzman and to Carol Strausser

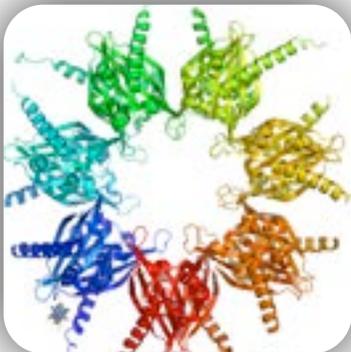
# Synthetic Strategies



Chemical  
Semisynthesis

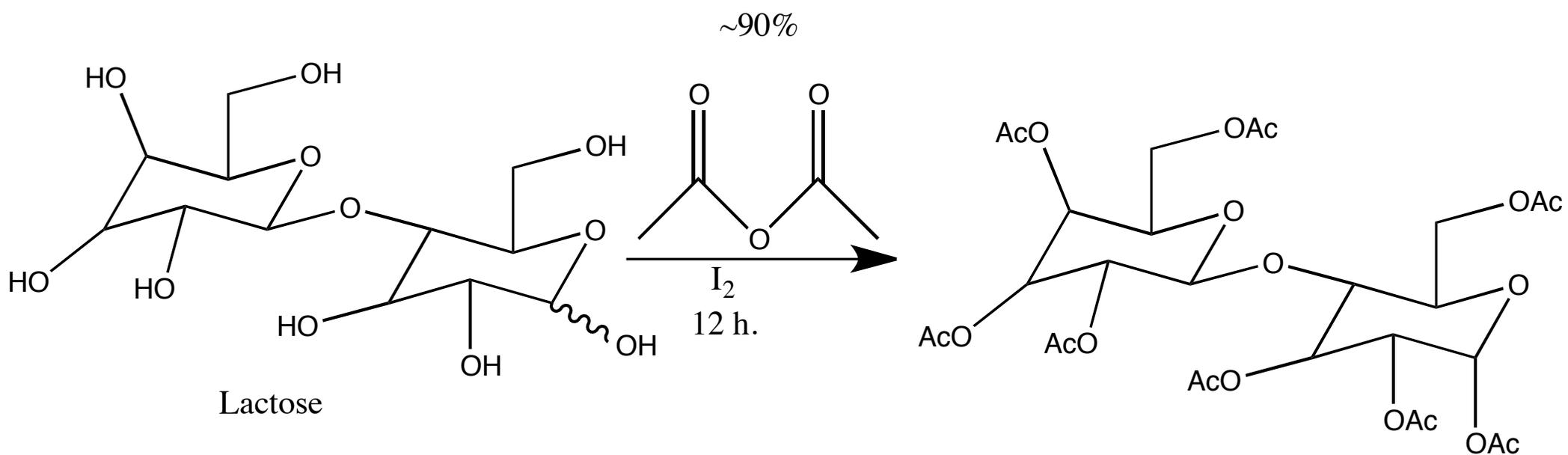
Chemoenzymatic  
Semisynthesis

600-02461516 [RF] © www.visualphotos.com

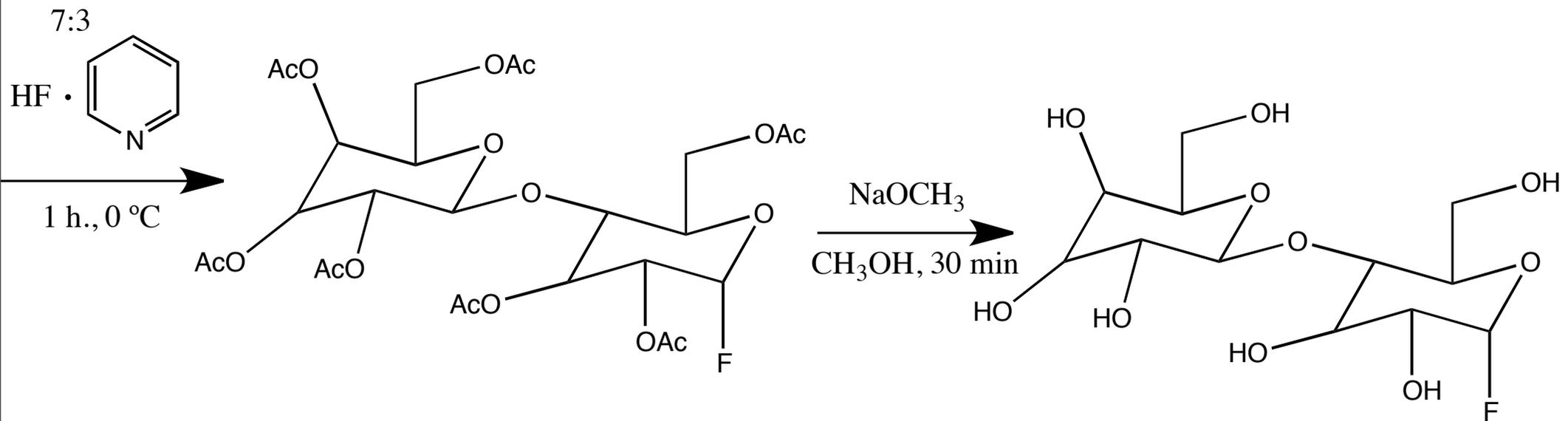


## Chemoenzymatic Semisynthesis

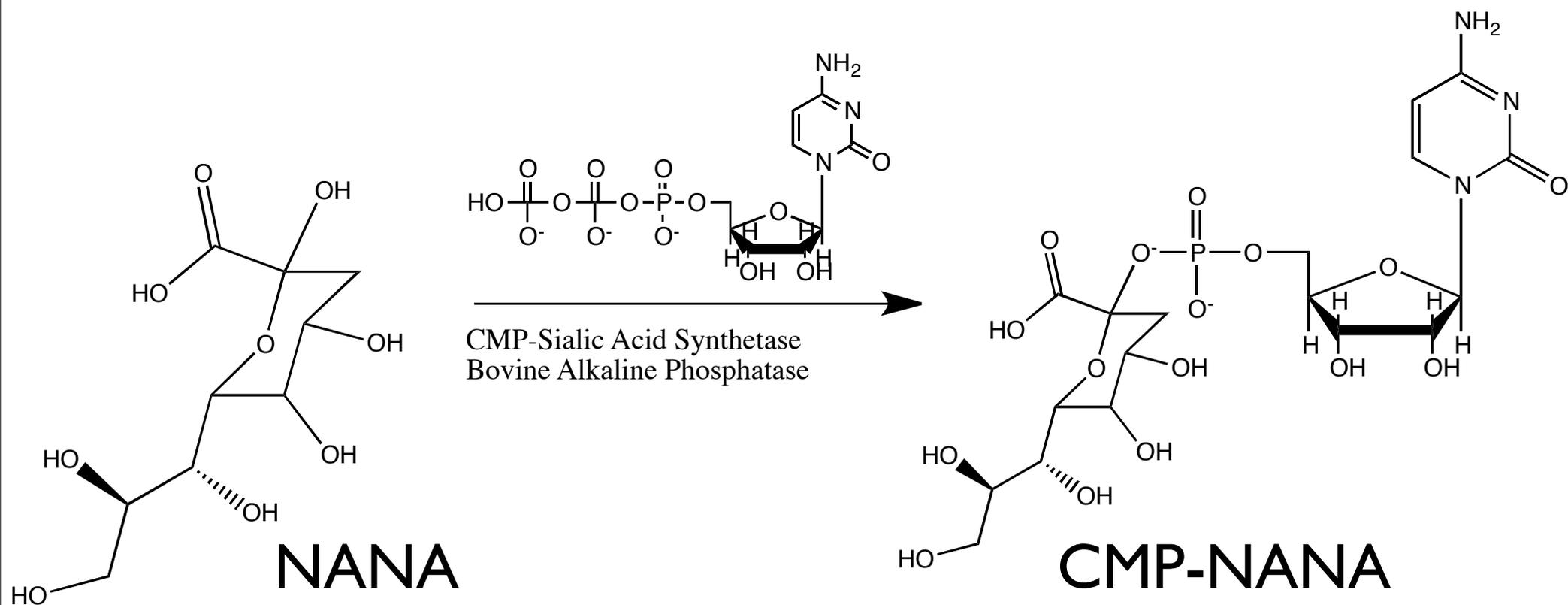
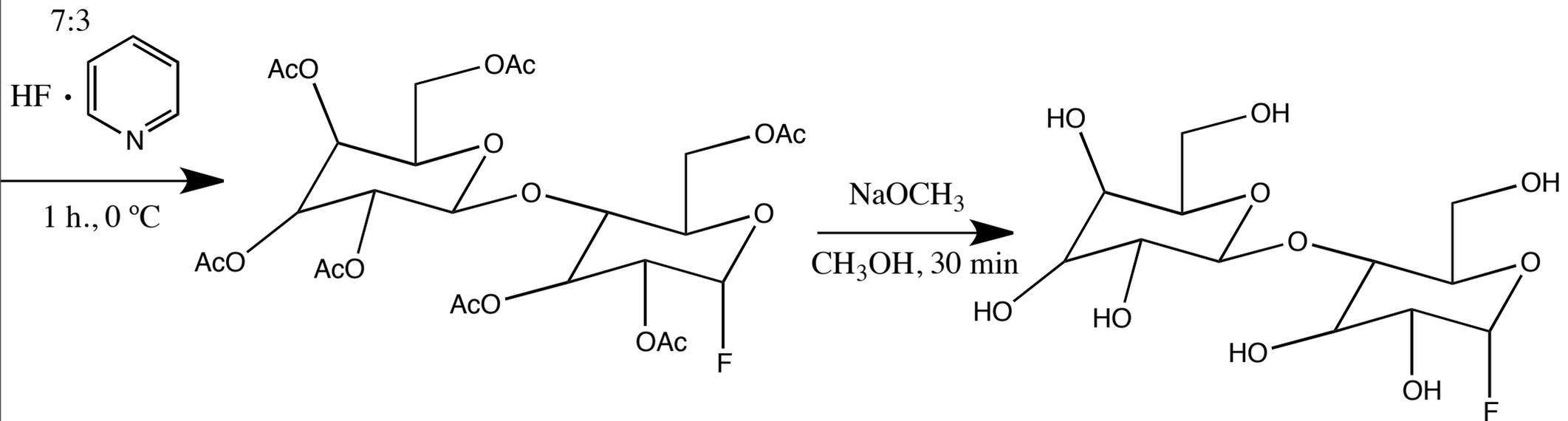
# Sugar



Ng, E. S. P. Master's Thesis. *U. British Columbia* **2005**.  
Rich, J. R.; Withers, S. G. *Angew. Chem. Int. Ed.* **2012**, *51*, 8640–8643.

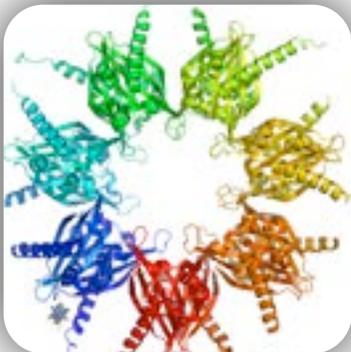


Ng, E. S. P. Master's Thesis. *U. British Columbia* **2005**.  
Rich, J. R.; Withers, S. G. *Angew. Chem. Int. Ed.* **2012**, *51*, 8640–8643.



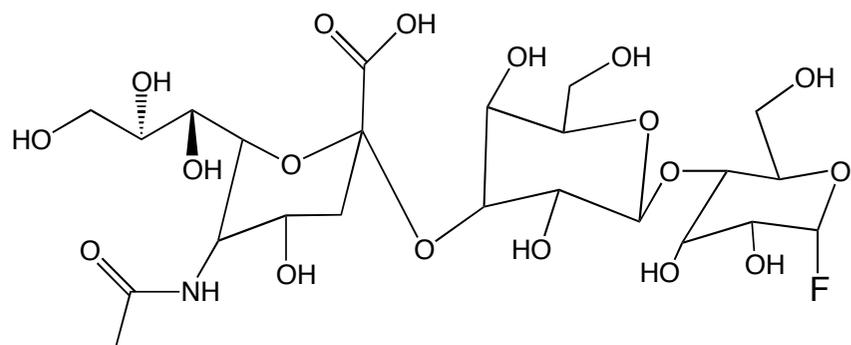
Ng, E. S. P. Master's Thesis. *U. British Columbia* **2005**.  
 Rich, J. R.; Withers, S. G. *Angew. Chem. Int. Ed.* **2012**, *51*, 8640–8643.



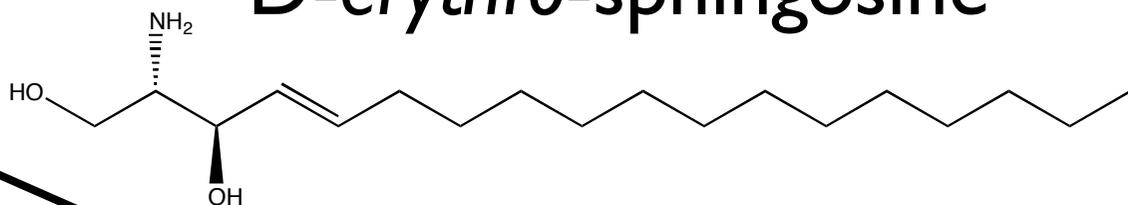


# Chemoenzymatic Semisynthesis: Coupling

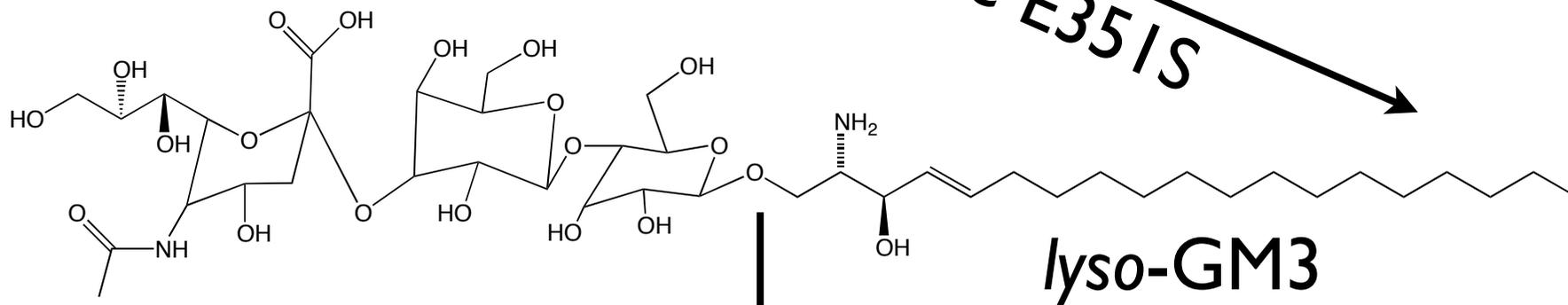
3'-SL Fluoride



D-erythro-sphingosine



EGCase E351S



lyso-GM3

Acylation

GM3

Vaughan, M. D.; Johnson, K.; Defrees, S.; Tang, X.; Warren, R. A. J.;  
Withers, S. G. *J. Am. Chem. Soc.* **2006**, *128*, 6300–6301.