Synthesis of Human Gpi Core Structure: Mass-Spectrometry as a Fundamental Tool

Barbara Richichi

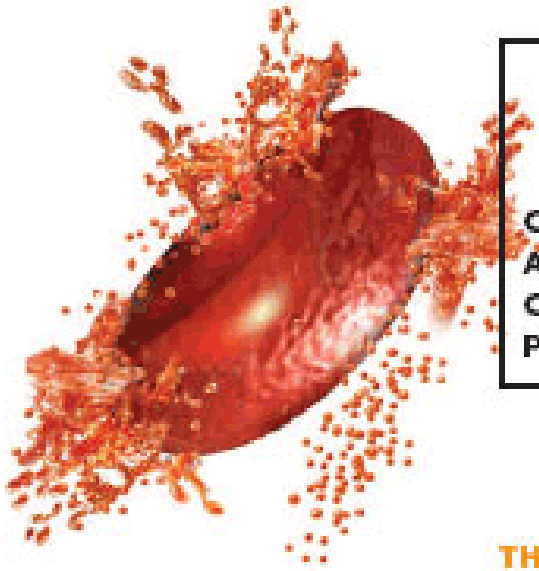
University of Florence
Department of Chemistry

PNH: *P*aroxysmal *N*octurnal *H*emoglobinuria

END ORGAN DAMAGE
BRAIN | LUNG | LIVER | GI | KIDNEY

IMPAIRED QoL
DISABLING FATIGUE | POOR PHYSICAL FUNCTION | PAIN

ANEMIA
TRANSFUSIONS | FATIGUE | DYSPNEA | HAEMOGLOBINURIA



SMOOTH MUSCLE DYSTONIA
ABDOMINAL PAIN | DYSPHAGIA | ERECTILE DYSFUNCTION

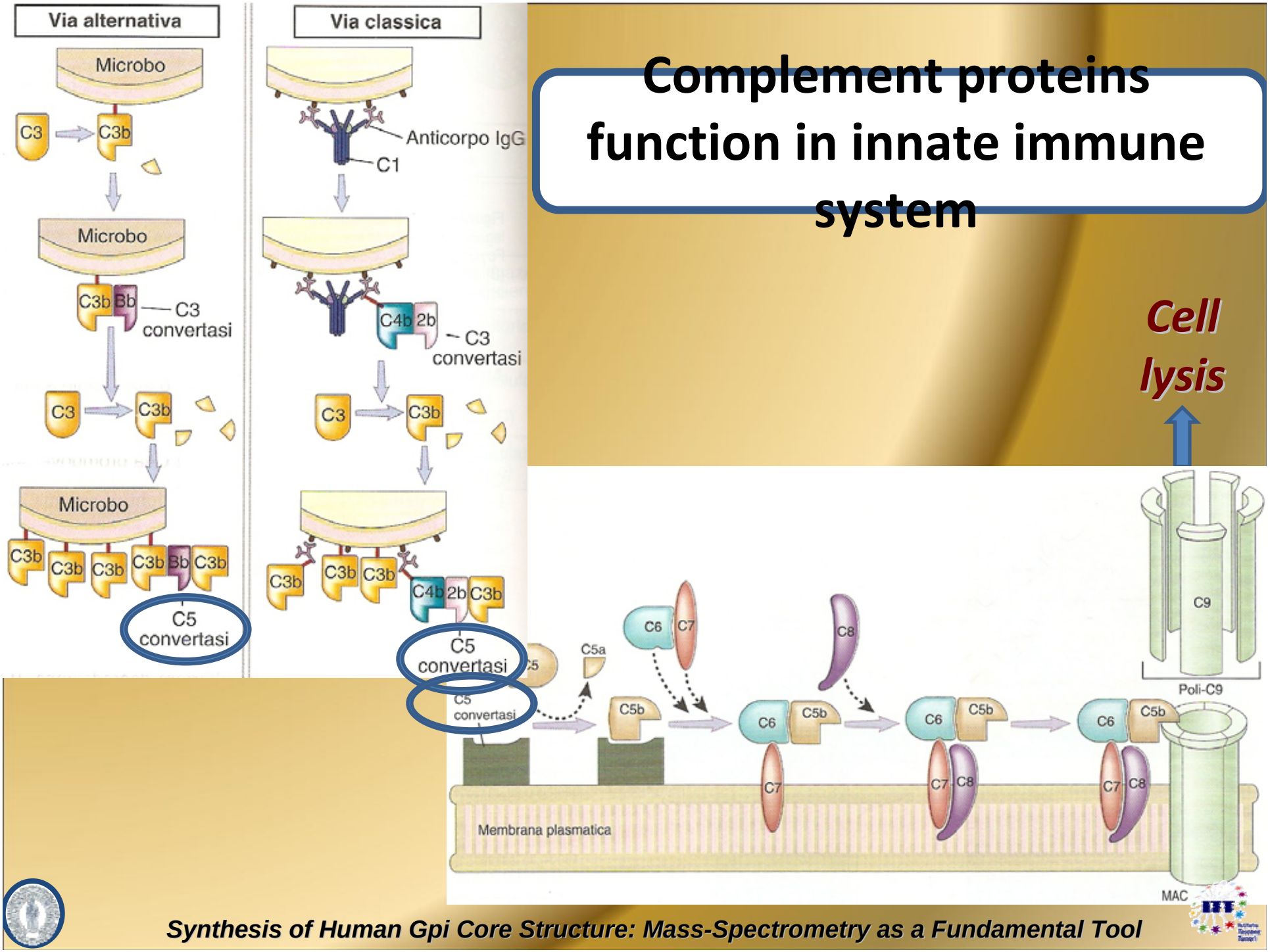
THROMBOSIS
VENOUS:
DVT | LIVER | DERMAL | CEREBRAL | MESENTERIC
ARTERIAL:
CVA | MI

CLINICAL SIGNS AND SYMPTOMS CAN BE PROGRESSIVE

Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare disorder characterized by intravascular hemolysis and hemoglobinuria.

PNH is the only hemolytic anemia caused by an acquired genetic mutation resulting in a intrinsic defect in the cell membrane of hematopoietic stem cell.

Complement proteins function in innate immune system



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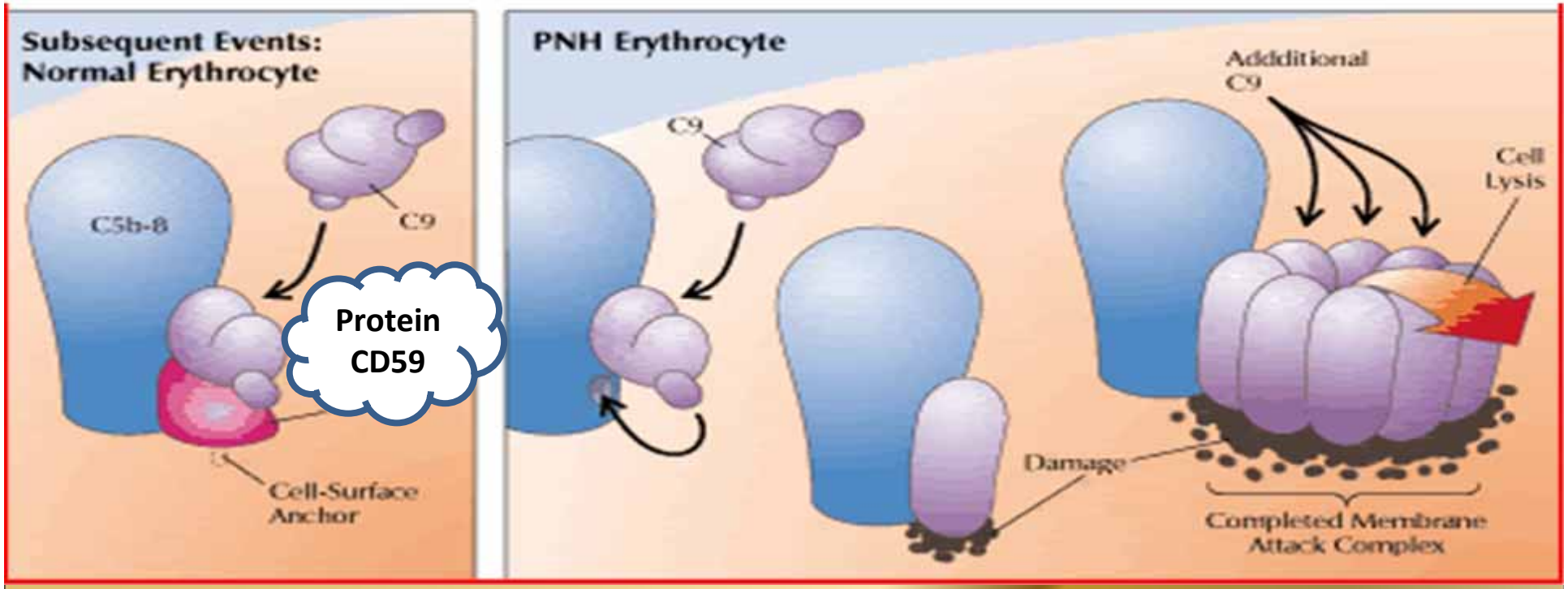
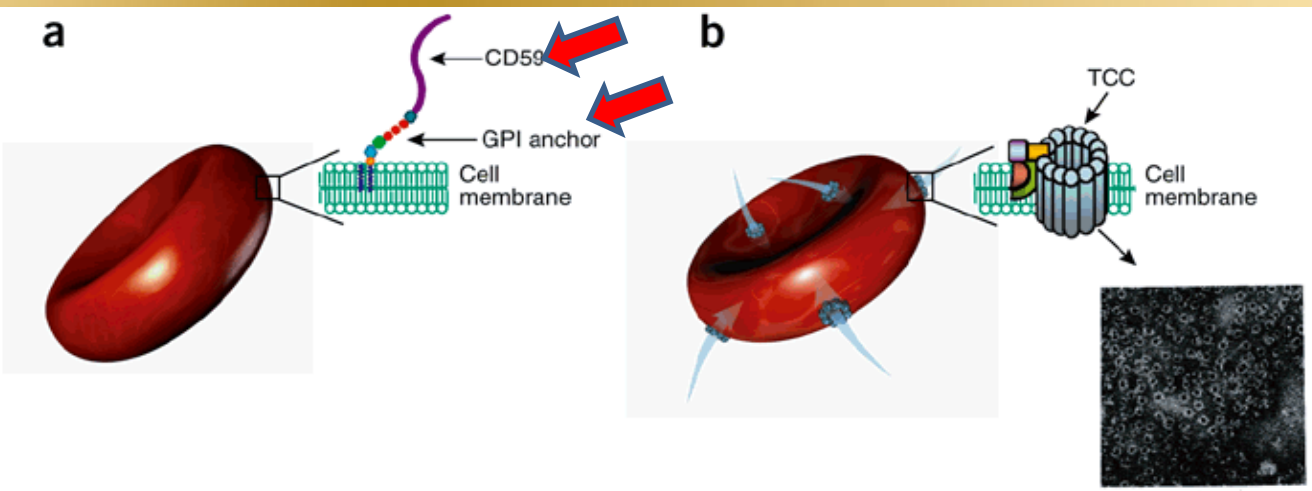
Complement regulation

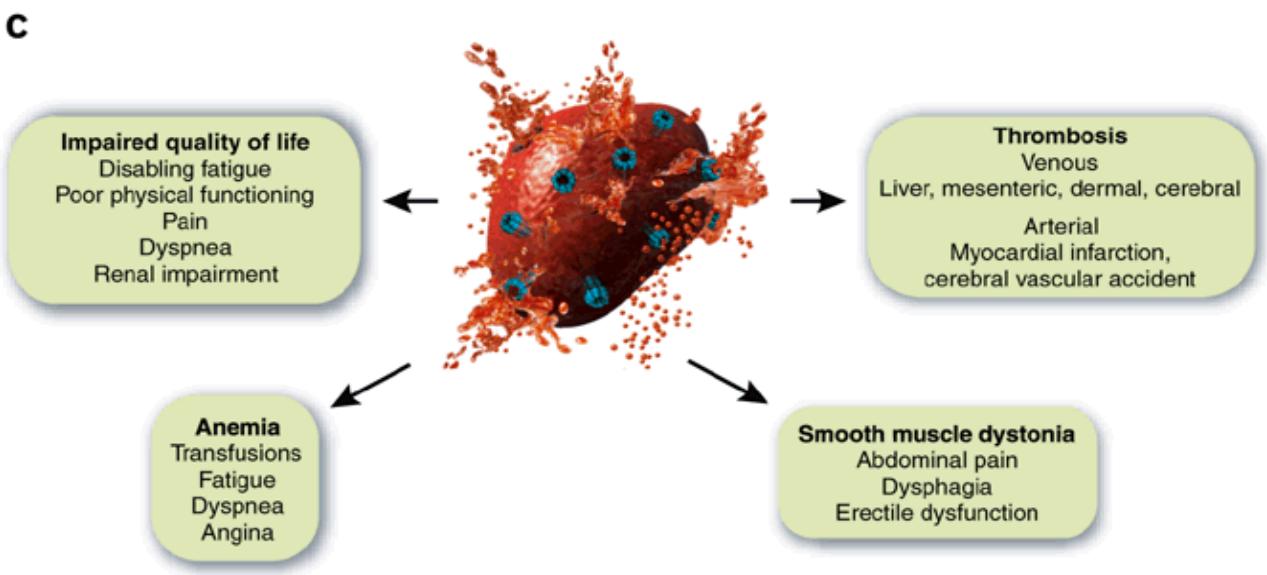
On cell membrane there are protein that inhibits the cytolytic action of complement



The complement-inhibitory function of CD59 derives from its capacity to interact with the C8 and C9 components of the cytolytic membrane attack complex to prevent insertion of C9 into the membrane

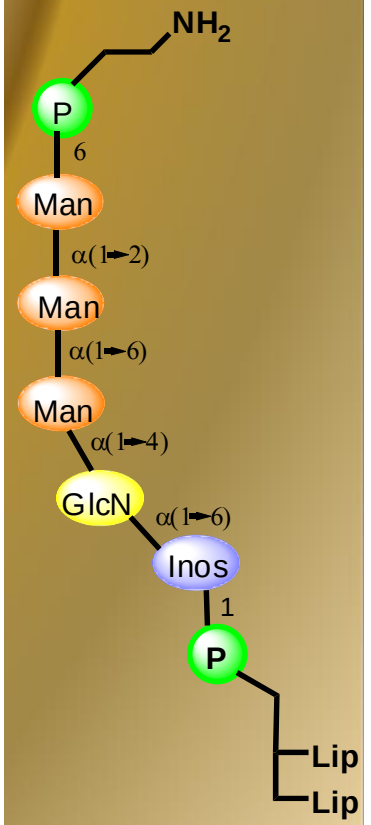
PNH: *P*aroxysmal *n*octurnal *h*emoglobinuria





Rosse W.F., Ware R.E., *Blood*, 86(9), **1995**, 3277

GPI anchor



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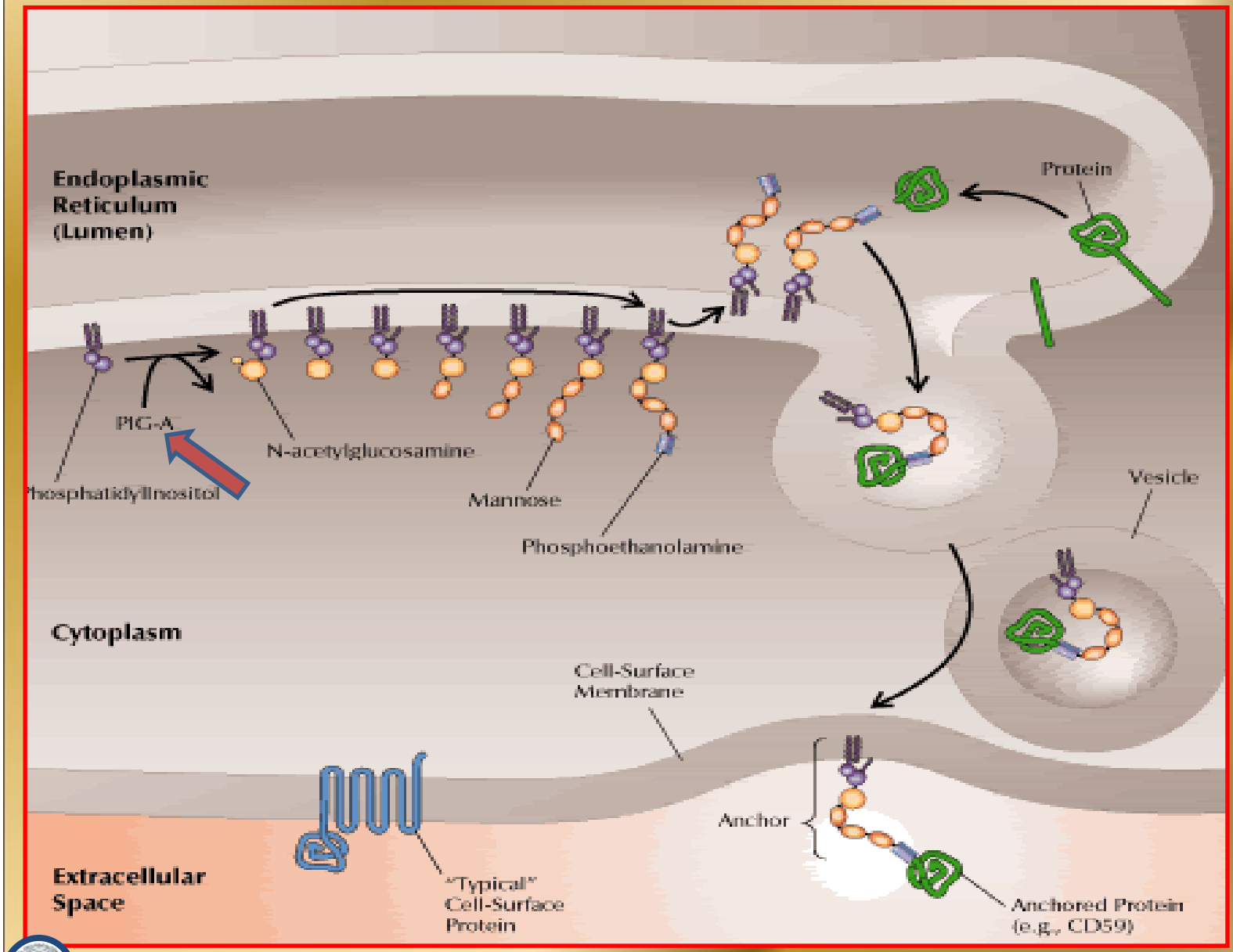
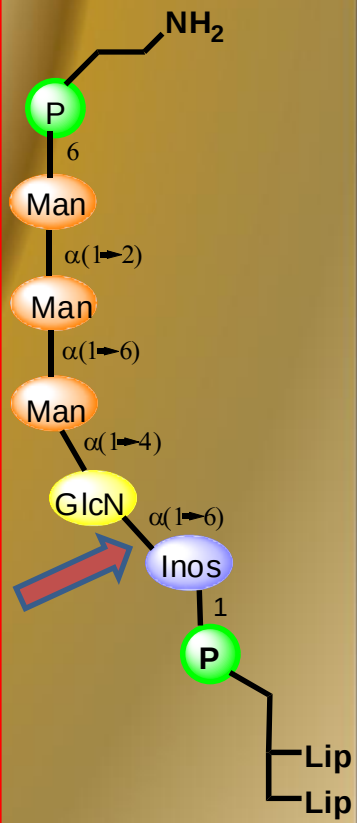
Biosynthesis of GPi





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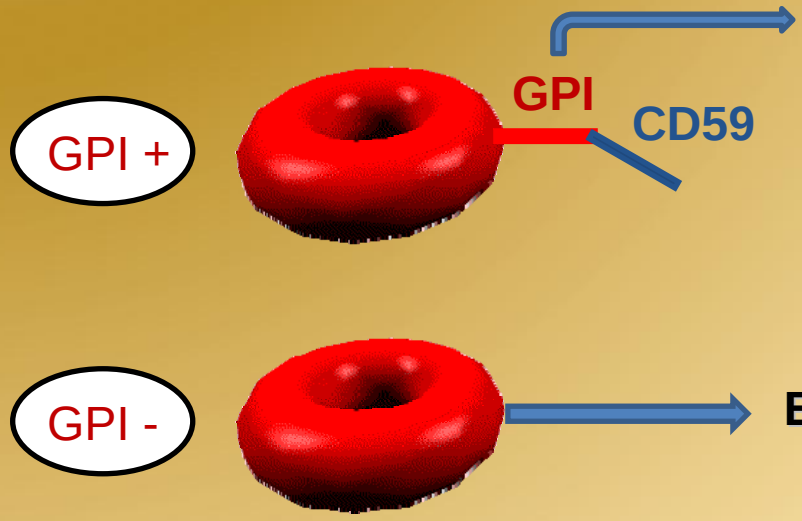
Paroxysmal Nocturnal Hemoglobinuria (PNH) is characterized by a clonal expansion of one or more hematopoietic stem cell.

Autoimmune process

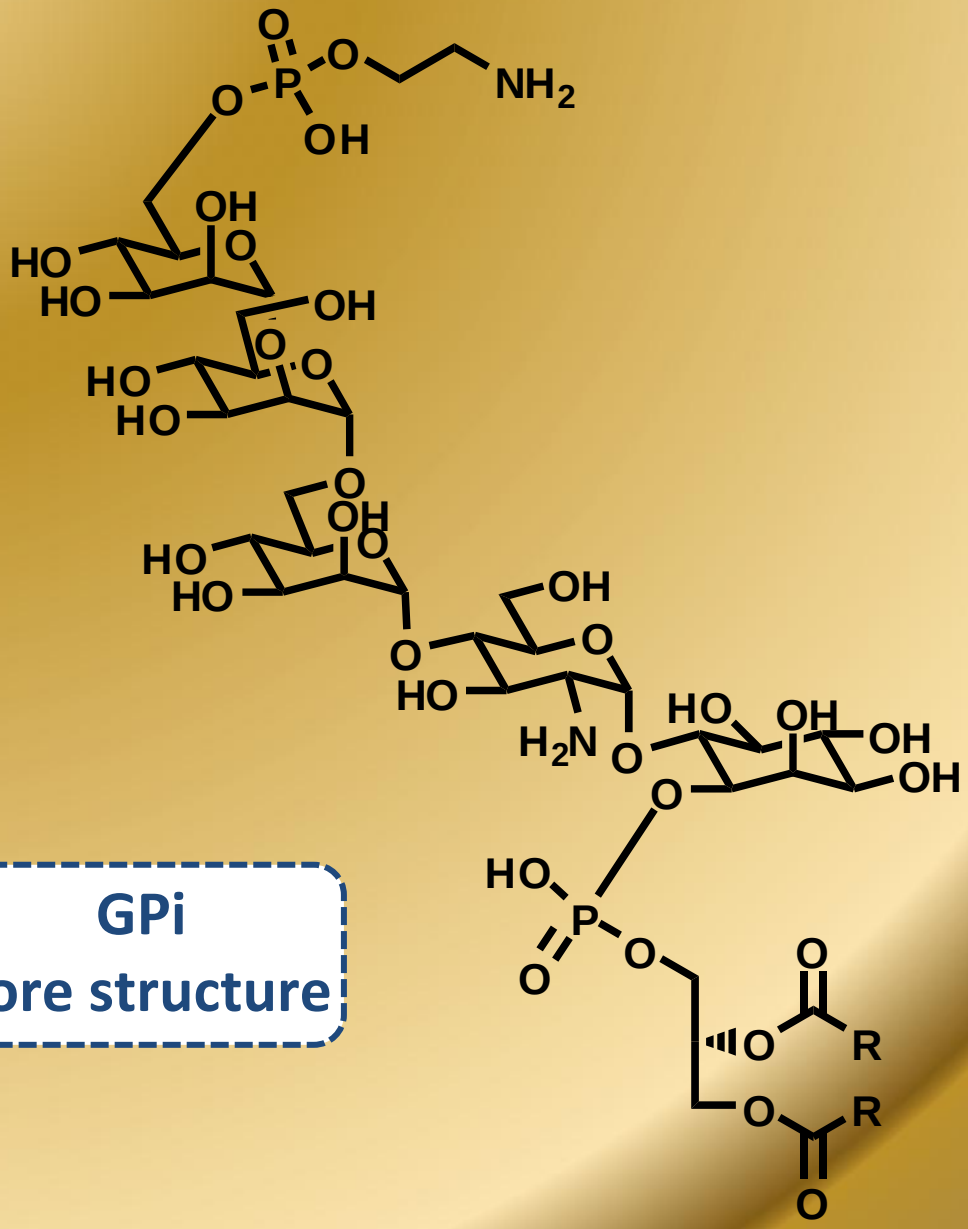


Luzzatto L. et al., *Blood*, 109(11), **2007**, 3277

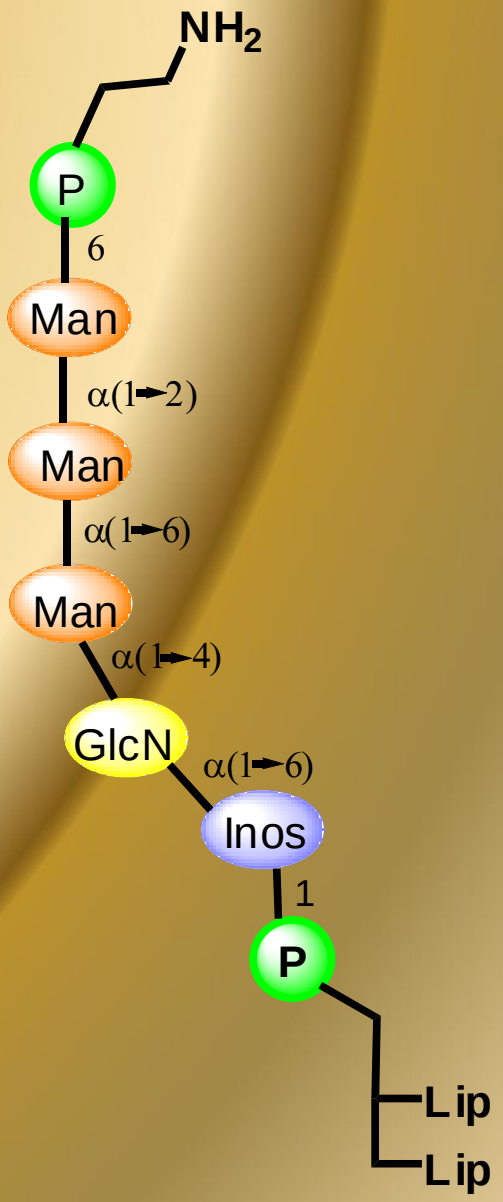
HYPHOTESIS

The expansion of the GPi$^-$ red blood cell population in PNH is due to selective damage to normal hematopoiesis madiated by an autoimmune attack against a non peptide antigen that could be the GPi anchor itself

Glycosyl Phosphatidylinositols o GPi



GPi
Core structure



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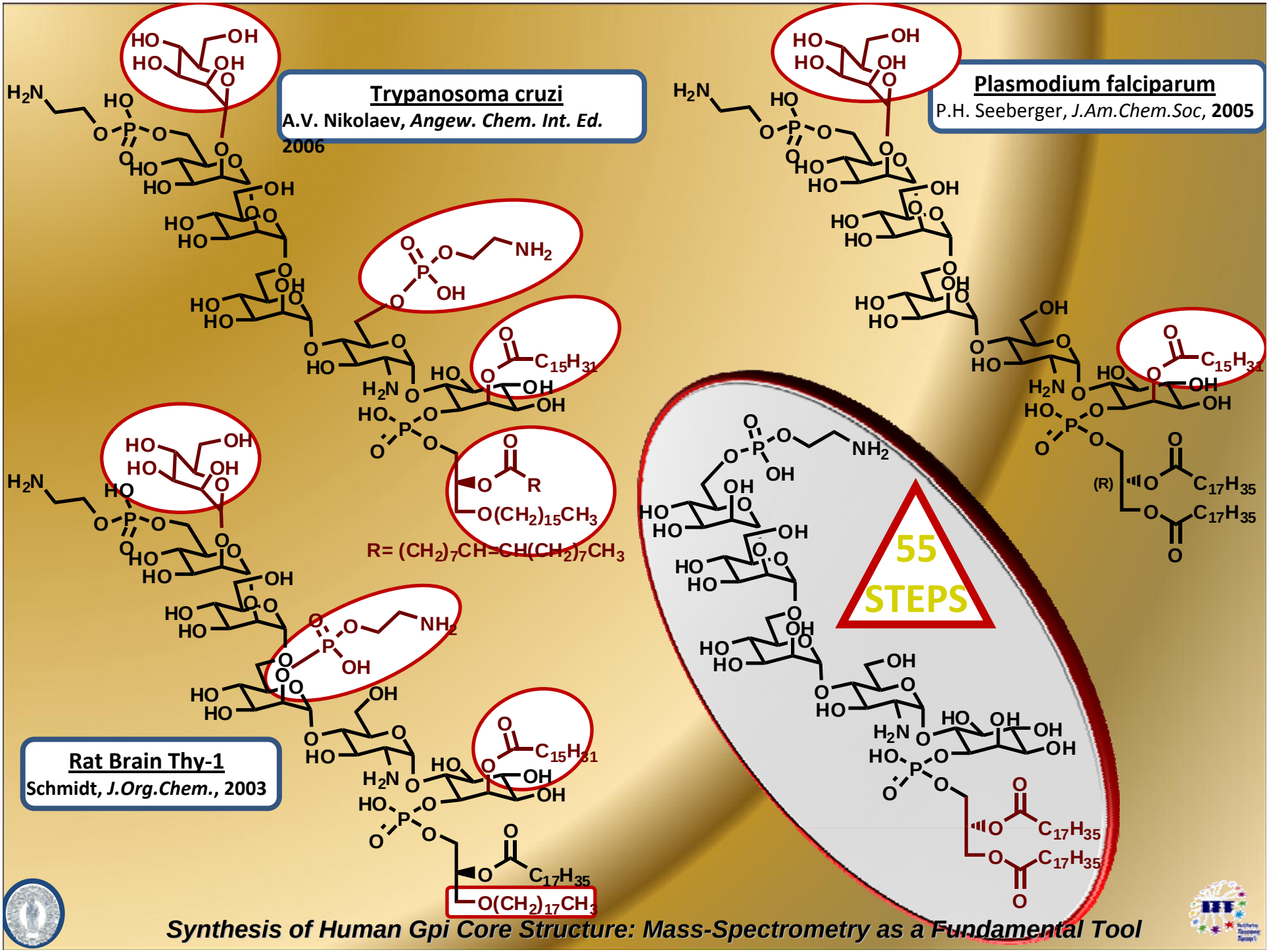
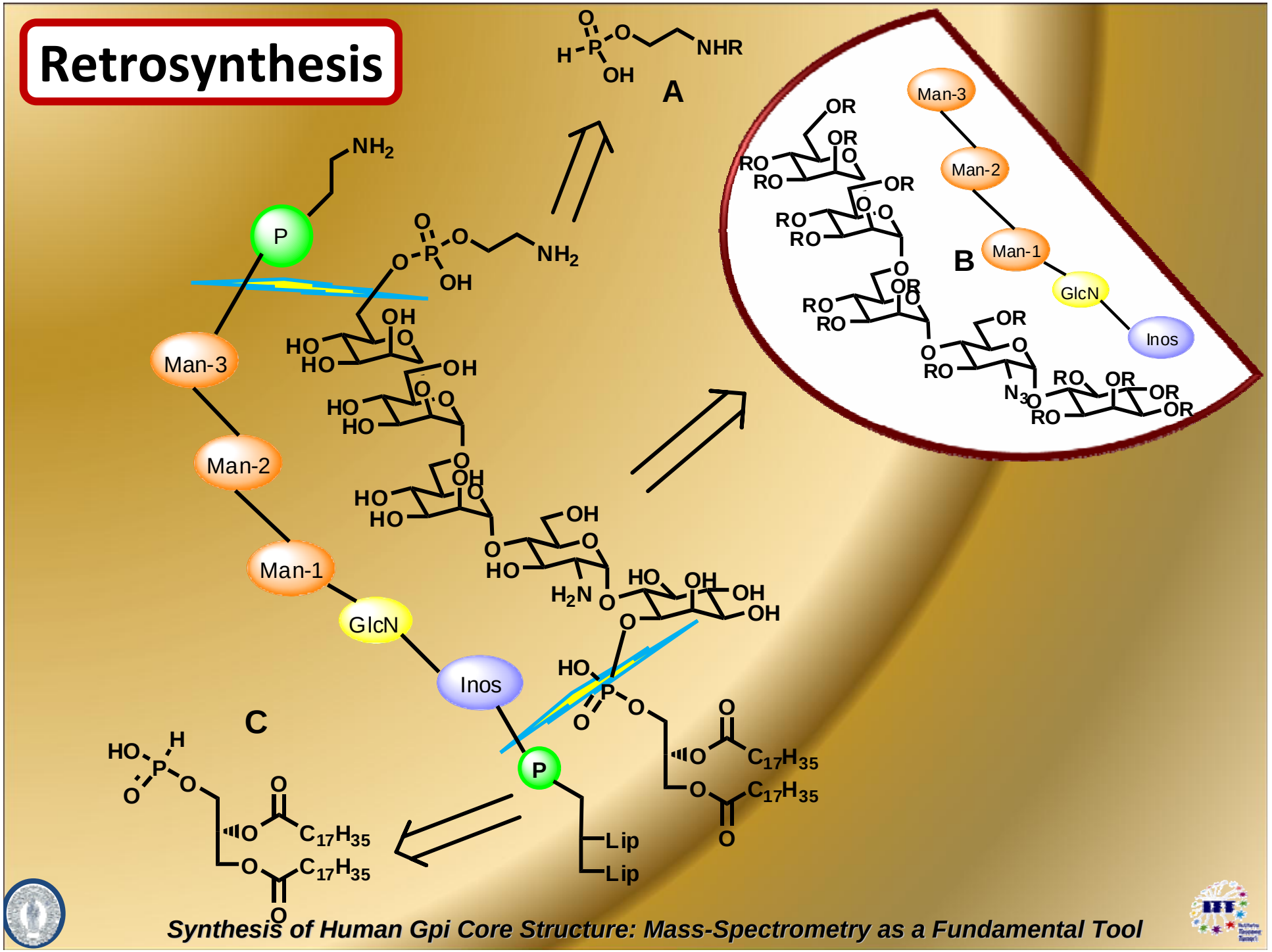

Trypanosoma cruzi
A.V. Nikolaev, Angew. Chem. Int. Ed. 2006
Plasmodium falciparum
P.H. Seeberger, J.Am.Chem.Soc, 2005
Rat Brain Thy-1
Schmidt, J.Org.Chem., 2003
R= (CH2)7CH=CH(CH2)7CH3
55 STEPS
Synthesis of Human Gpi Core Structure: Mass-Spectrometry as a Fundamental Tool


Retrosynthesis
A
B
C
Man-3
Man-2
Man-1
GlcN
Inos
P
Lip
Lip
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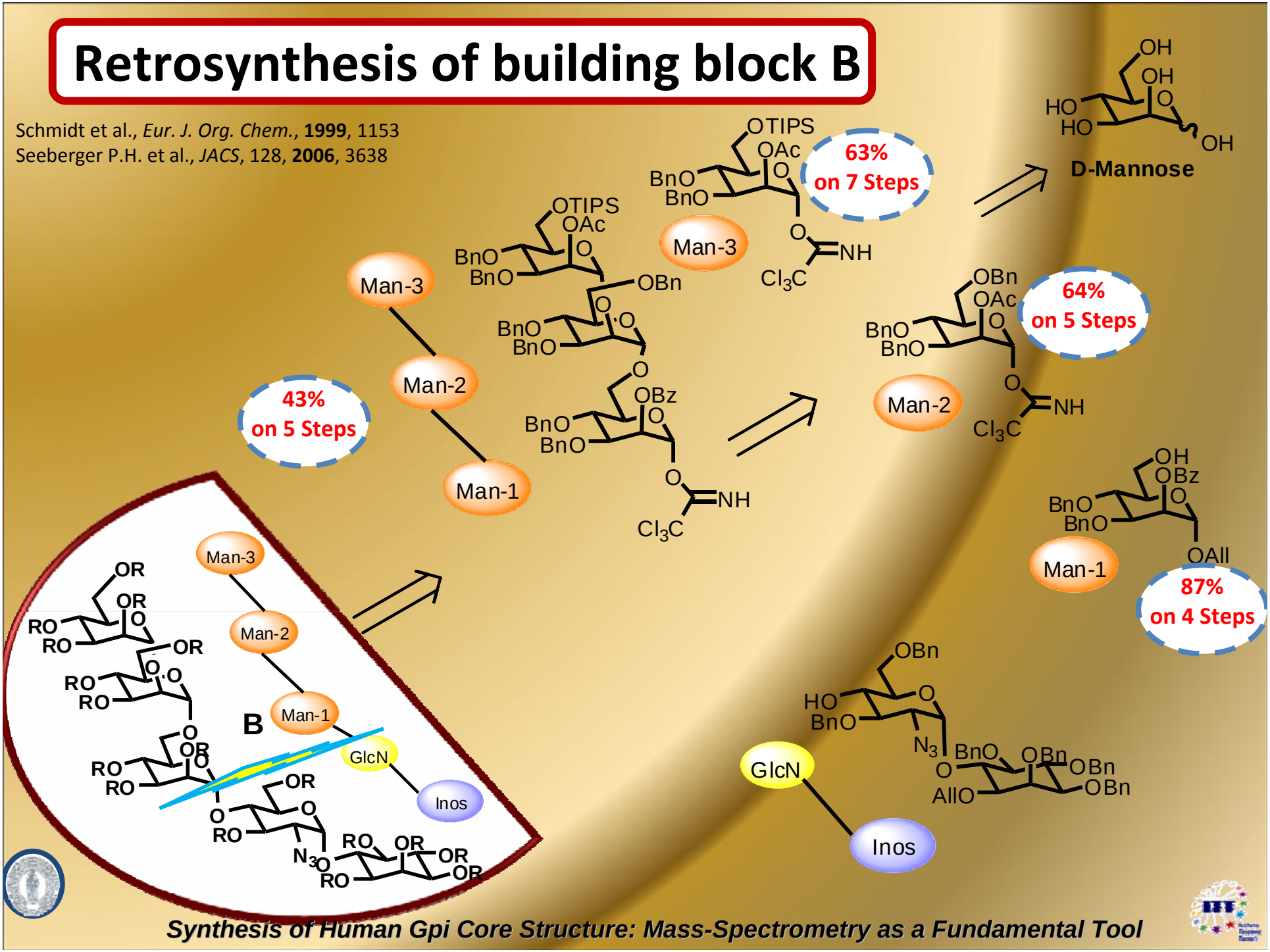
Retrosynthesis of building block B

Schmidt et al., *Eur. J. Org. Chem.*, **1999**, 1153
Seeberger P.H. et al., *JACS*, 128, **2006**, 3638



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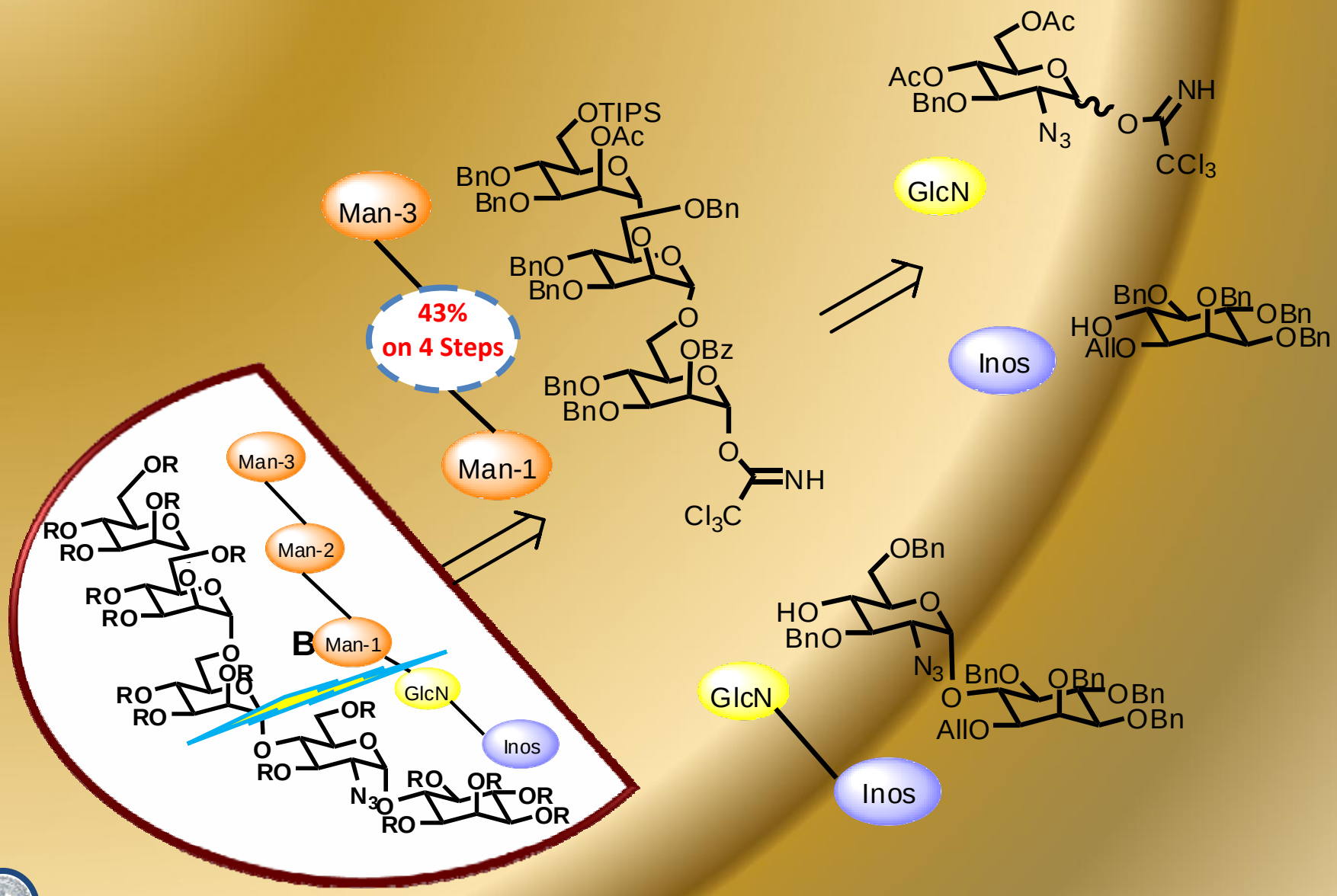
Retrosynthesis of building block B



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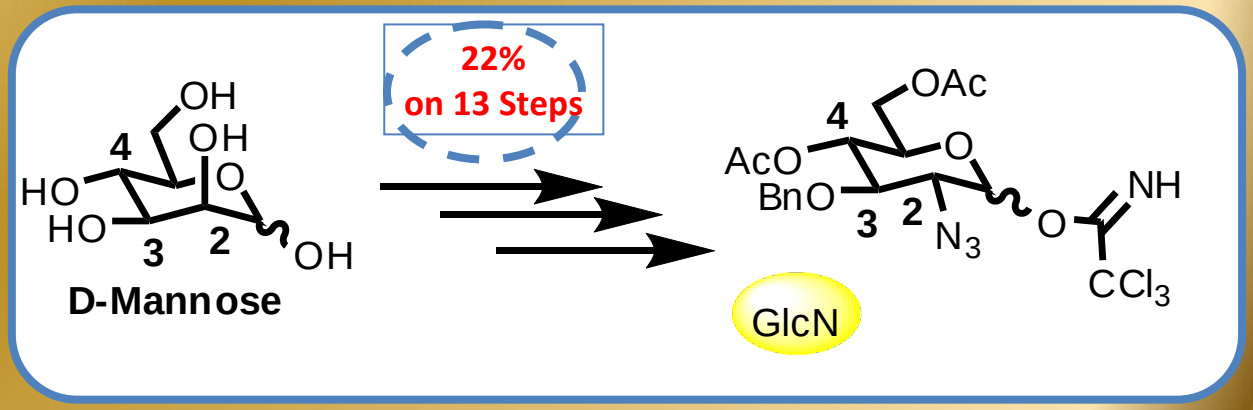
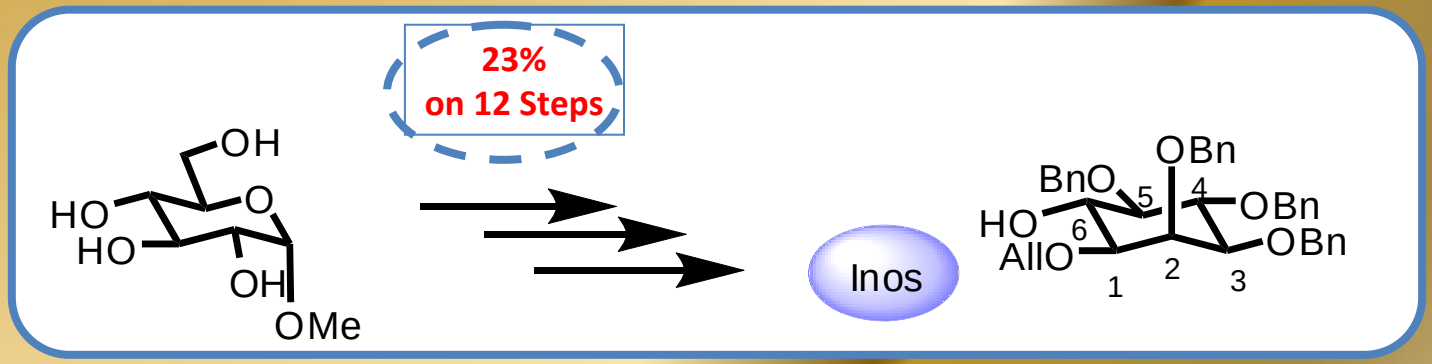
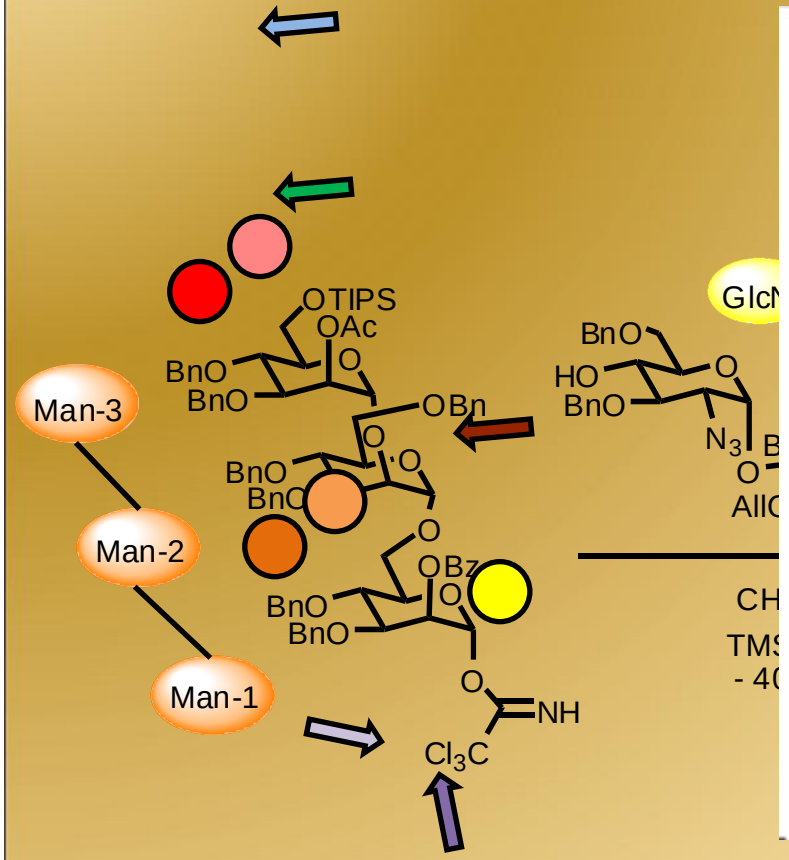
Retrosynthesis of glucosazide



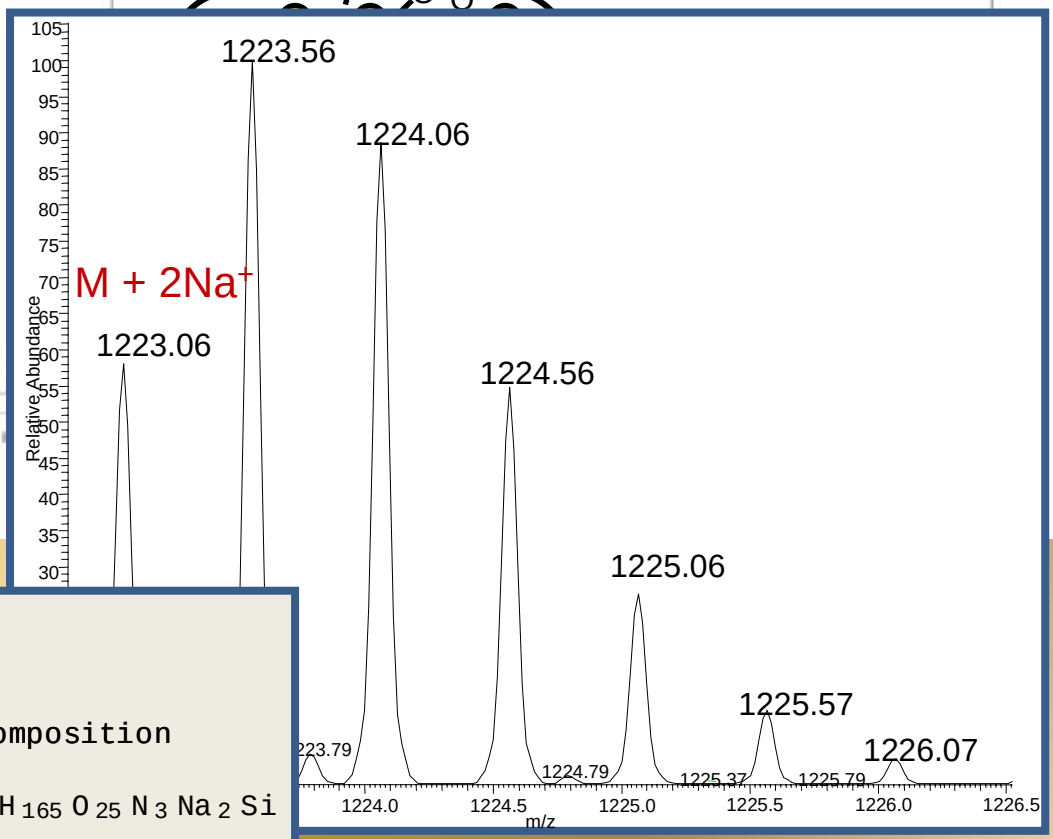
Retrosynthesis of myo-inositol



Synthesis of building block B



^{1}H-NMR (400MHz)




```
Elemental composition search on mass 1223.06

m/z=  1218.06-1228.06
    m/z      | Theo. Mass | Delta (ppm) | RDB equiv. | Composition
 1223.05902  | 1223.06429 |    -4.31    |    67.0    | C147 H165 O25 N3 Na2 Si
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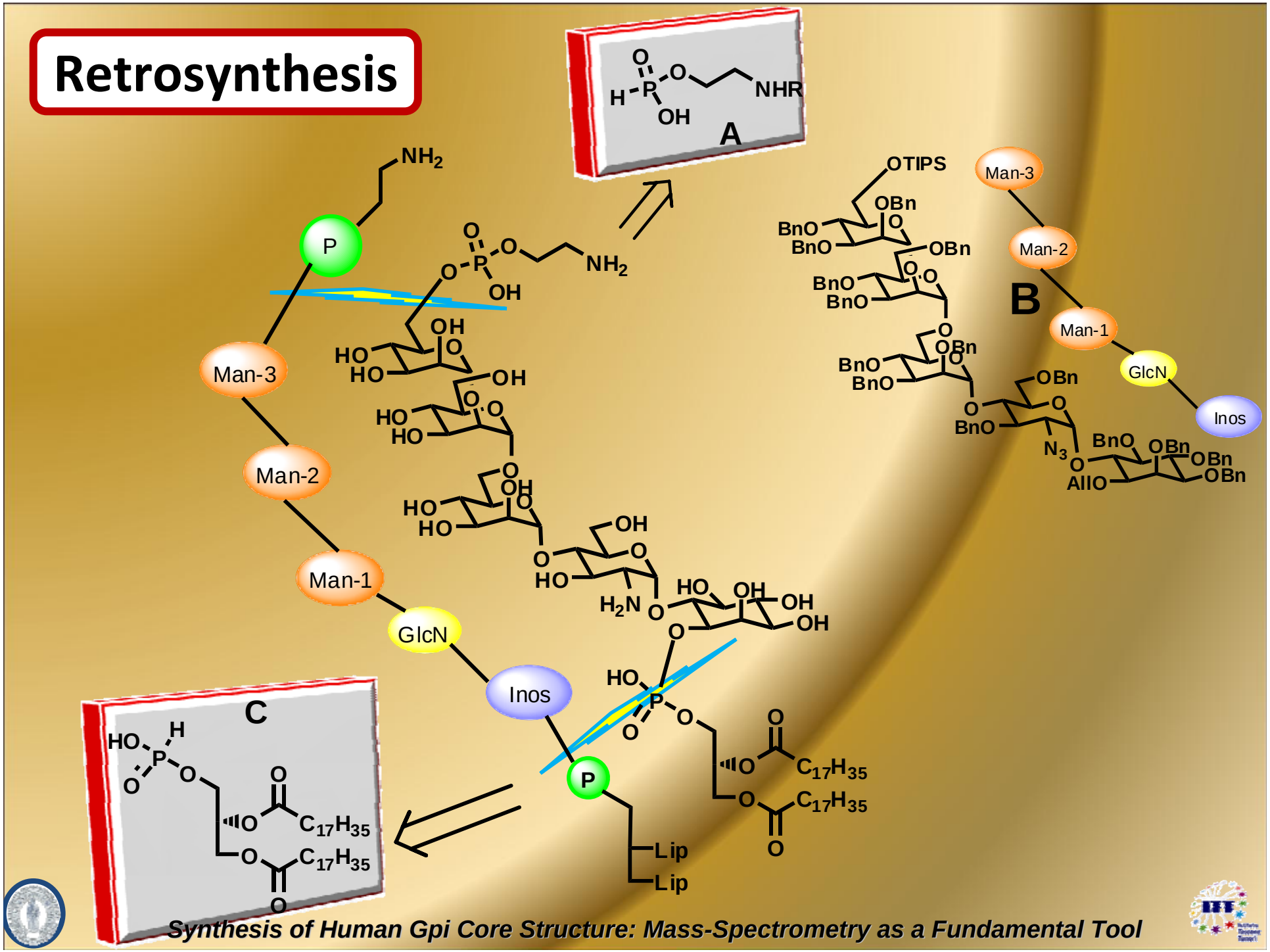
Retrosynthesis
A
B
C
Man-3
Man-2
Man-1
GlcN
Inos
P
Lip
Lip
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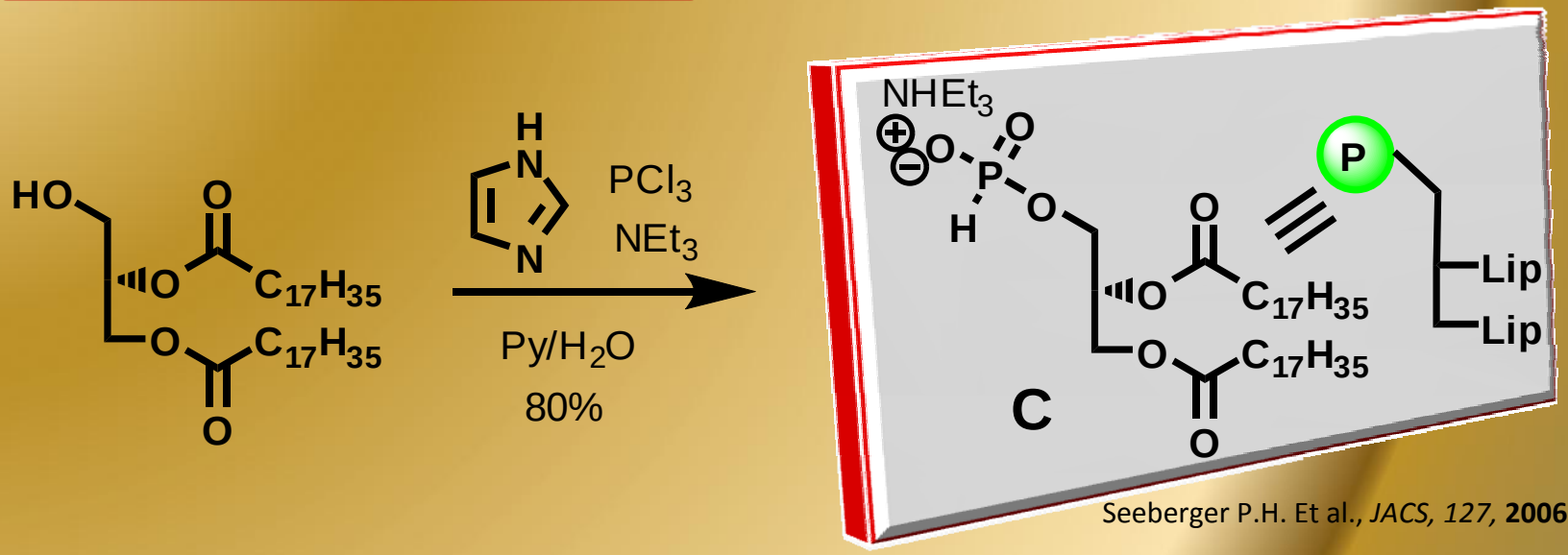
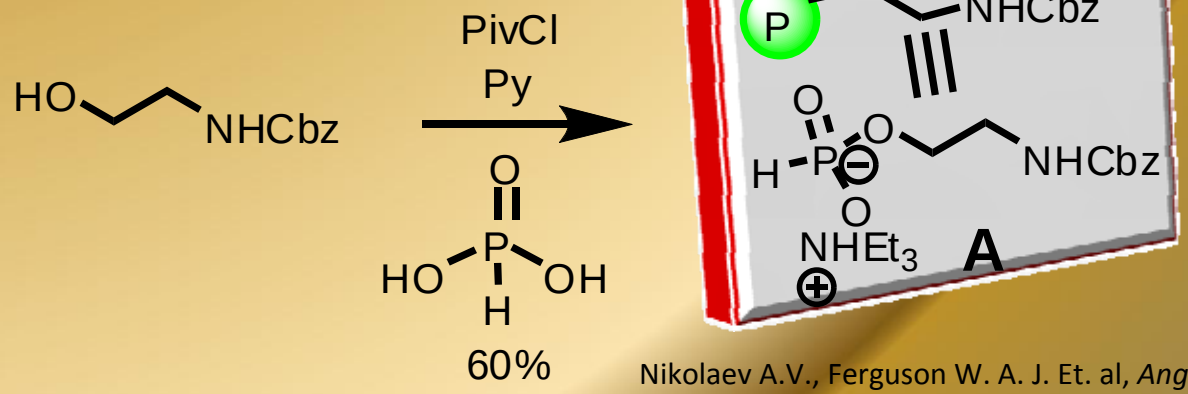
Synthesis of A and C



Seeberger P.H. Et al., *JACS, 127,* **2006**, 5004

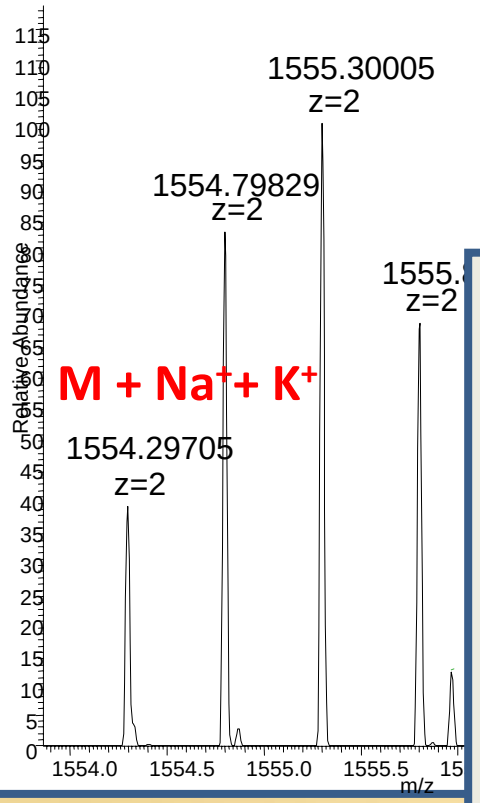


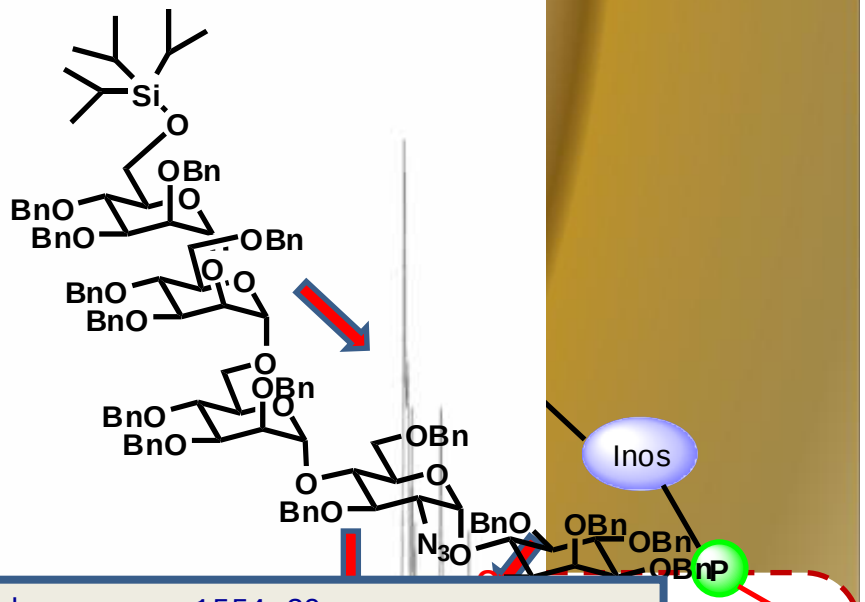
Nikolaev A.V., Ferguson W. A. J. Et. al, *Angew. Che. Int. Ed.,*45, **2006**, 468

Synthesis of GPi core structure

^{1}H-NMR (400MHz)

^{31}P-NMR





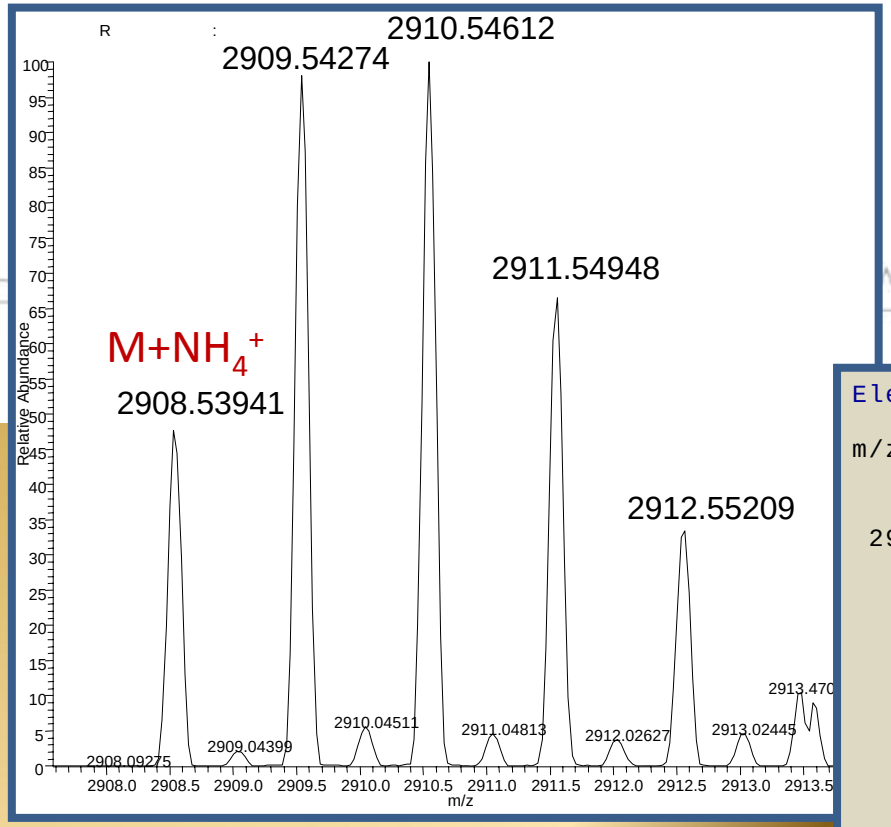
Elemental composition search on mass 1554.30

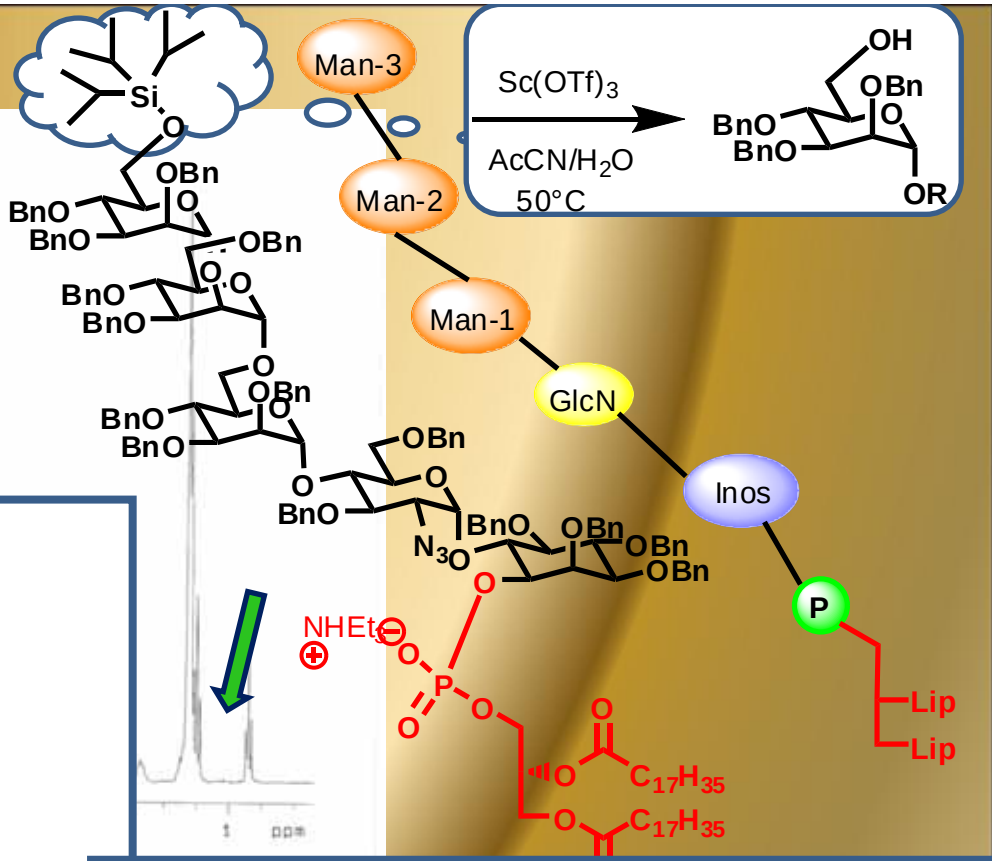
m/z= 1549.30-1559.30

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1554.29705	1554.29679	0.17	63.5	$C_{180}H_{238}O_{35}N_2KNaPSi$
	1554.29664	0.27	68.0	$C_{184}H_{235}O_{35}NKNaSi$
	1554.29813	-0.70	68.0	$C_{183}H_{236}O_{32}N_3KNaPSi$
	1554.29880	-1.13	67.5	$C_{185}H_{238}O_{33}KNaPSi$
	1554.29522	1.18	68.5	$C_{184}H_{234}O_{34}N_2KNaP$
	1554.30151	-2.87	68.0	$C_{185}H_{236}O_{34}NKNaP$
	1554.29252	2.92	68.0	$C_{184}H_{236}O_{33}NKNaPSi$
	1554.30225	-3.35	68.0	$C_{183}H_{235}O_{34}N_3KNaSi$
	1554.30292	-3.78	67.5	$C_{185}H_{237}O_{35}KNaSi$
	1554.30308	-3.88	63.0	$C_{181}H_{240}O_{35}NKNaPSi$

Synthesis of GPi core structure

^{1}H-NMR (400MHz)





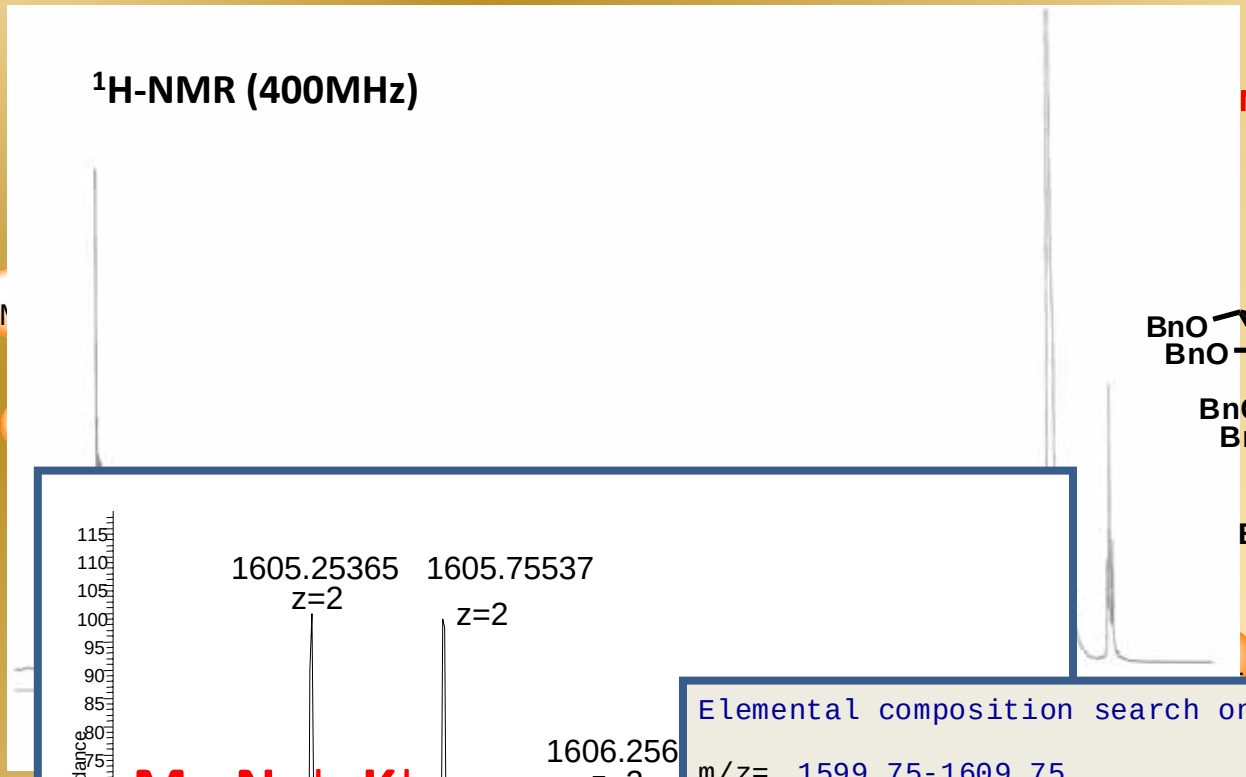
Elemental composition search on mass 2908.54

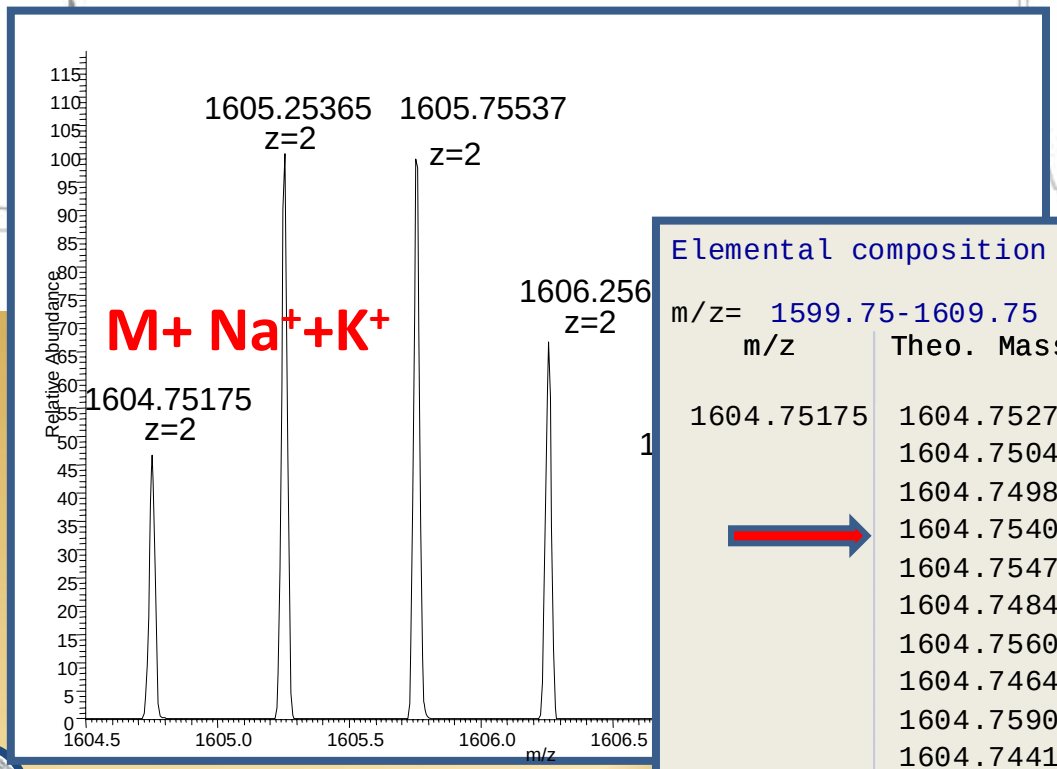
m/z= 2903.54-2913.54

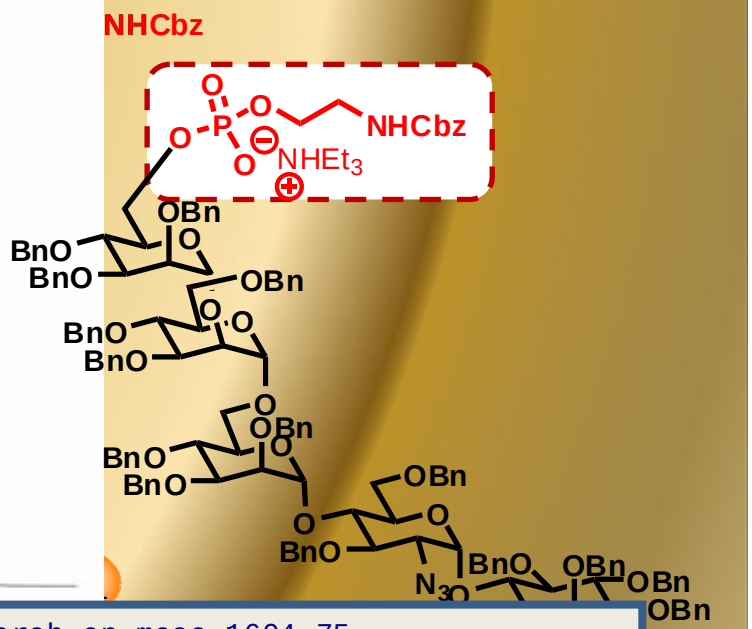
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
2908.53941	2908.54129	-0.65	67.5	$C_{175}H_{219}O_{35}N_2$
	2908.53707	0.80	71.5	$C_{180}H_{220}O_{31}P$
	2908.53573	1.27	72.0	$C_{178}H_{218}O_{30}N_3P$
	2908.54397	-1.57	72.0	$C_{178}H_{217}O_{32}N_3$
	2908.54428	-1.68	67.5	$C_{174}H_{220}O_{32}N_4P$
	2908.53408	1.83	77.0	$C_{180}H_{213}O_{29}N_5$
	2908.54531	-2.03	71.5	$C_{180}H_{219}O_{33}$
	2908.54563	-2.14	67.0	$C_{176}H_{222}O_{33}NP$
	2908.53305	2.19	67.5	$C_{175}H_{220}O_{33}N_2P$
	2908.53274	2.29	72.0	$C_{179}H_{217}O_{33}N$

Synthesis of GPi core structure

^{1}H-NMR (400MHz)







Elemental composition search on mass 1604.75

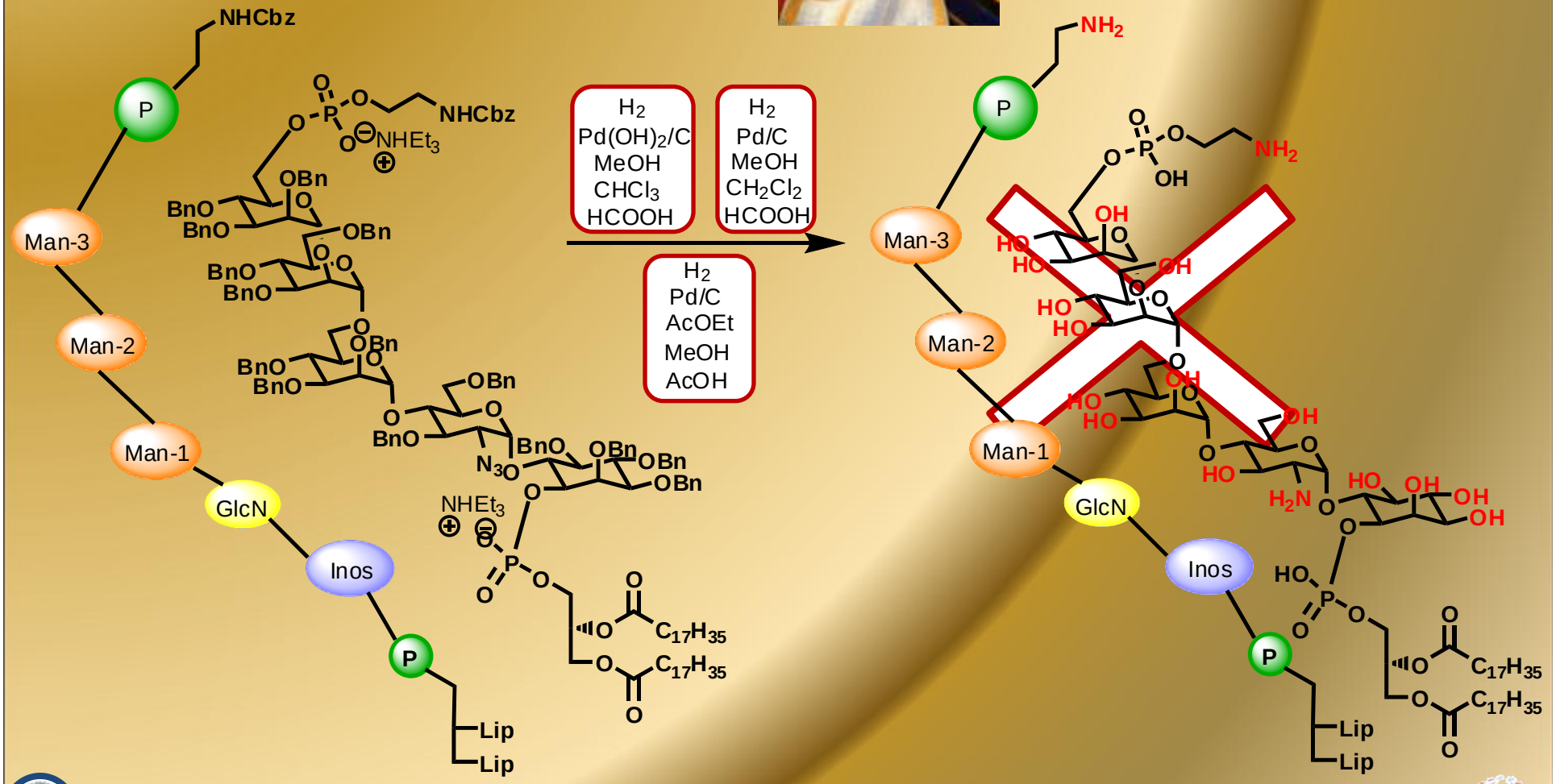
m/z= 1599.75-1609.75

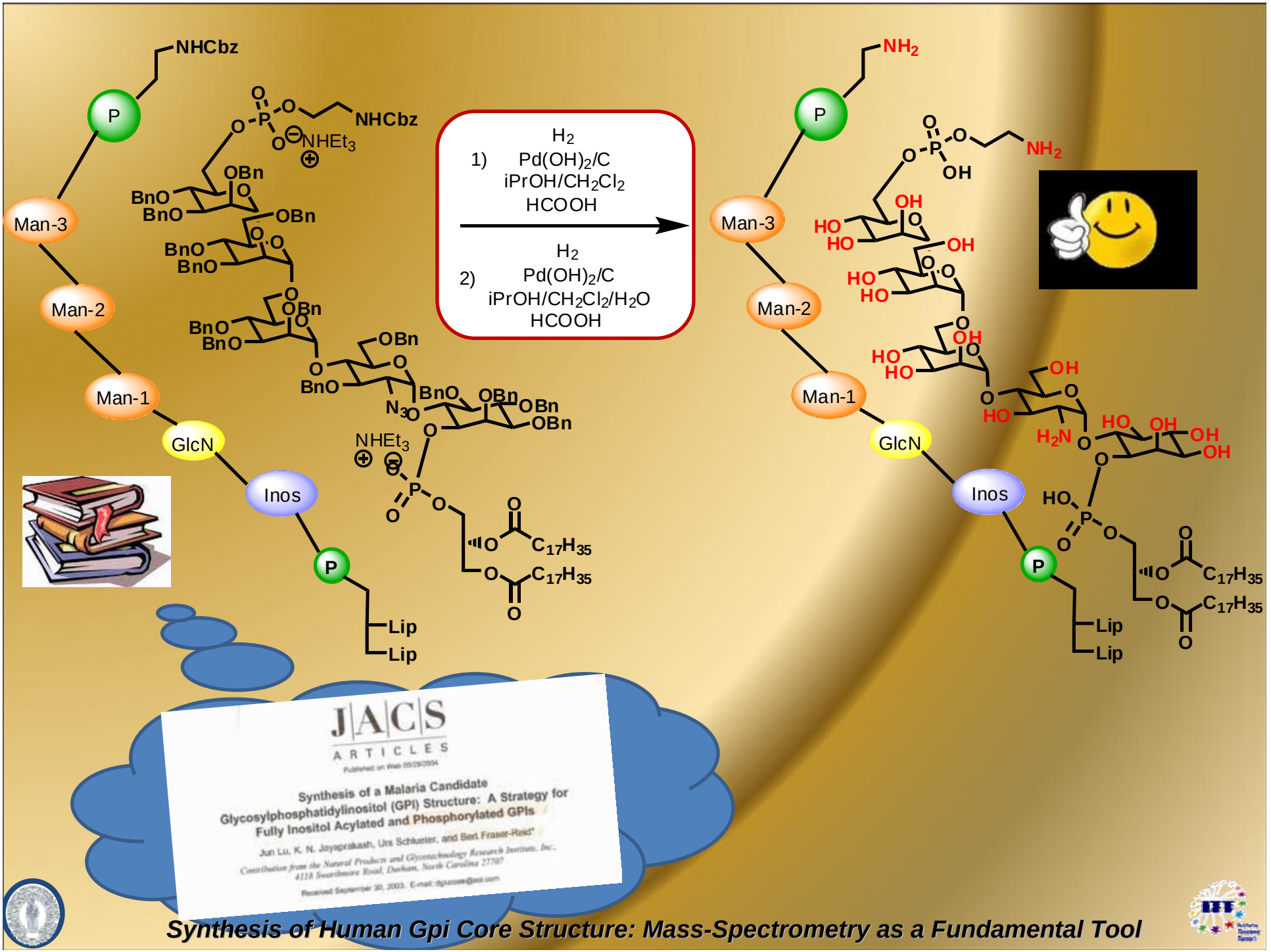
m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1604.75175	1604.75273	-0.61	68.5	$C_{181}H_{230}O_{40}N_3KNaP_2$
	1604.75047	0.80	77.0	$C_{190}H_{228}O_{36}KNaP_2$
	1604.74980	1.22	77.5	$C_{188}H_{226}O_{35}N_3KNaP_2$
	1604.75407	-1.45	73.0	$C_{184}H_{228}O_{37}N_4KNaP_2$
	1604.75474	-1.87	72.5	$C_{186}H_{230}O_{38}NKNaP_2$
	1604.74846	2.05	73.0	$C_{185}H_{228}O_{38}N_2KNaP_2$
	1604.75608	-2.70	77.0	$C_{189}H_{228}O_{35}N_2KNaP_2$
	1604.74644	3.31	69.0	$C_{180}H_{228}O_{40}N_4KNaP_2$
	1604.75902	-4.53	68.0	$C_{182}H_{232}O_{40}N_2KNaP_2$
	1604.74418	4.72	77.5	$C_{189}H_{226}O_{36}NKNaP_2$

$C_{17}H_{35}$

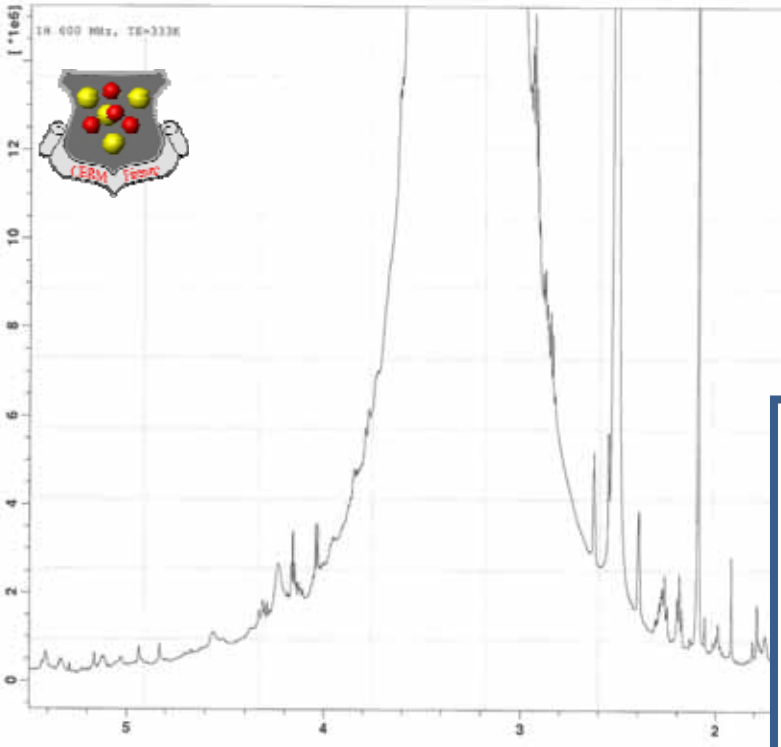
$C_{17}H_{35}$

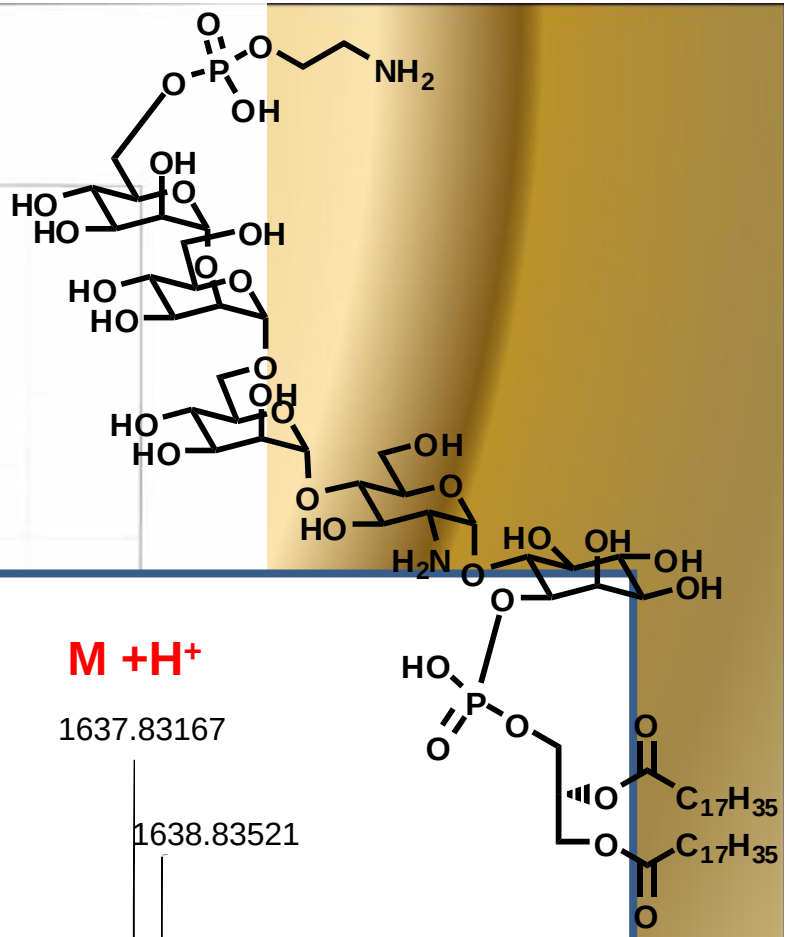
The last step.....

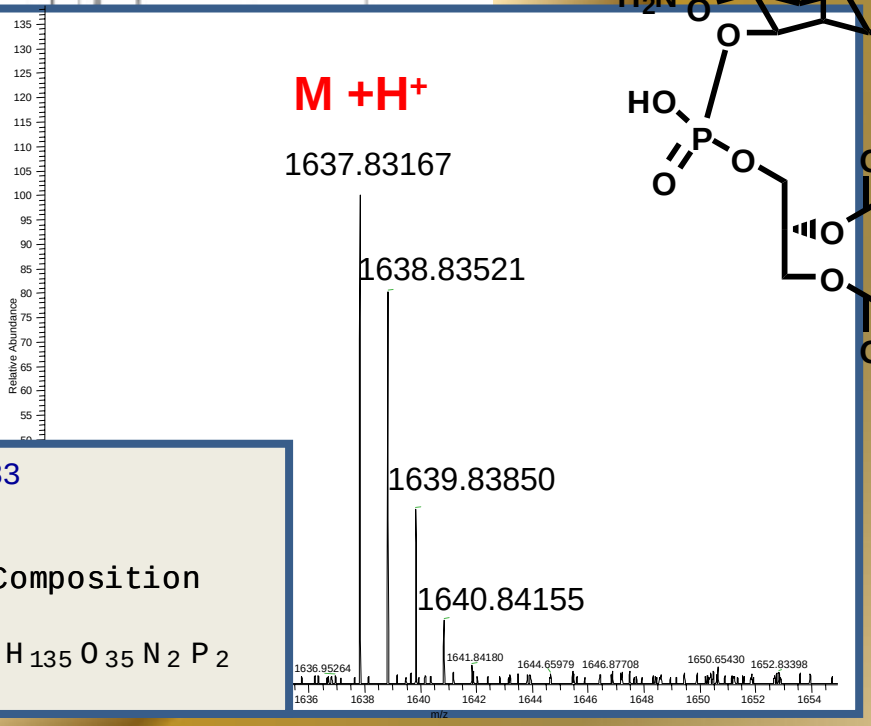



NHCbz
P
NHCbz
NHEt3
OBn
BnO
Man-3
Man-2
Man-1
GlcN
Inos
Lip
Lip
H2
1) Pd(OH)2/C
iPrOH/CH2Cl2
HCOOH
H2
2) Pd(OH)2/C
iPrOH/CH2Cl2/H2O
HCOOH
NH2
OH
HO
H2N
C17H35
C17H35
J|A|C|S
ARTICLES
Synthesis of a Malaria Candidate
Glycosylphosphatidylinositol (GPI) Structure: A Strategy for
Fully Inositol Acylated and Phosphorylated GPIs
Jun Lu, K. N. Jayaprakash, Urs Schlueter, and Bert Fraser-Reid*
Synthesis of Human Gpi Core Structure: Mass-Spectrometry as a Fundamental Tool

1H-NMR (600MHz)







Elemental composition search on mass 1637.83

m/z= 1632.83-1642.83

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
1637.83167	1637.83151	0.10	6.5	$C_{71}H_{135}O_{35}N_2P_2$

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Acknowledgements

University of Florence

Prof.ssa Cristina Nativi

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Prof. Lucio Luzzatto
Dott. Rosario Notaro

Centro Interdipartimentale di Spettrometria di Massa

Prof. Gloriano Moneti
Dott. Giuseppe Pieraccini

University of Padua

Dott.ssa Roberta Seraglia

NMR

CERM-University of Florence

Menarini Ricerche

Dott. Antonio Triolo