

NEW ASPECTS OF GLYCOSIDE BOND FORMATION – FROM THE GENOME TO THE PROTEOME AND THEN TO THE GLYCOME?

RICHARD R. SCHMIDT

The organizing Committee / Reko Lehtilä, CSC et al.

"We would appreciate ... if you could speak

- not only about your recent work, but also ... about
- how your research projects have developed,
- how your research group is organized,
- what type of collaboration you have."

"We would greatly appreciate if you could give us your view

- on the recent development of this field and also
- how you see its future."

IMPORTANT ASPECTS OF RECENT WORK ON GLYCOCONJUGATE SYNTHESIS – FROM THE GENOME TO THE PROTEOME AND THEN TO THE GLYCOME?

RICHARD R. SCHMIDT

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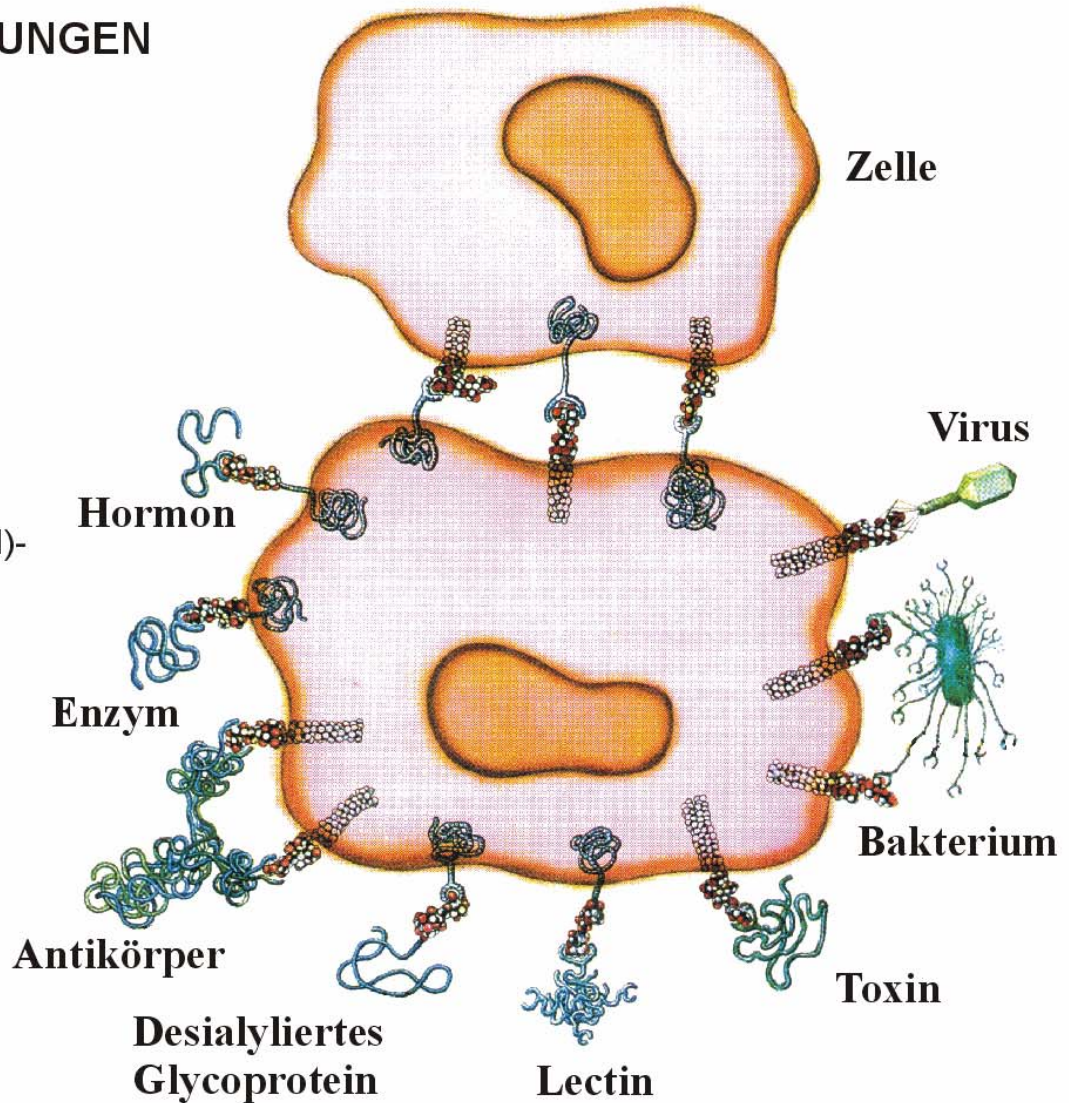
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University of Konstanz (State Baden-Württemberg)
Deutsche Forschungsgemeinschaft (DFG)
Fonds der Chemischen Industrie
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Ludwig Institute for Cancer Research, NY
Various Industrial Companies

KOHLLENHYDRAT-WECHSELWIRKUNGEN AUF DER ZELLOBERFLÄCHE

Andockstellen auf der Zelle sind:

- GLYCOSPHINGOLIPIDE / GANGLIOSIDE
- PROTEINE / GLYCOPROTEINE
- GLYCOSYLPHOSPHATIDYL INOSITOL (GPI)-
GEANKERTE GLYCOPROTEINE



Quelle: BioCarb Chemicals Catalogue 90

FUNCTIONS OF OLIGOSACCHARIDES AS CONSTITUENTS OF GLYCOCONJUGATES (GSL, GP, GPI)

S. Hakomori; A. Varki, al.

- (1) Structural, protecting, and stabilizing function
- (2) Specific receptors for (symbiotic, pathogenic) bacteria and parasites
- (3) Tuning/Fine-tuning of proteins (polysialylation of N-CAM, etc.)
- (4) Depot function on the cell surface (for instance, for growth factors)
- (5) Influence on the inter- and intracellular transport between cell compartments
- (6) Hormonal action (oligosaccharins in plants, etc.)
- (7) Cell-cell and cell-matrix recognition (selectins, etc.)

Are there any general principles?

- (1) The same structure can have different functions! – Time and space separated expression
- (2) "Junk"-oligosaccharides? – Defence against pathogens?
- (3) Intra- and interspecies variations in the glycosylation pattern (blood groups!)
- (4) Non reducing end sequences and unusual structures are primarily involved in specific functions.

A. Varki (1993):

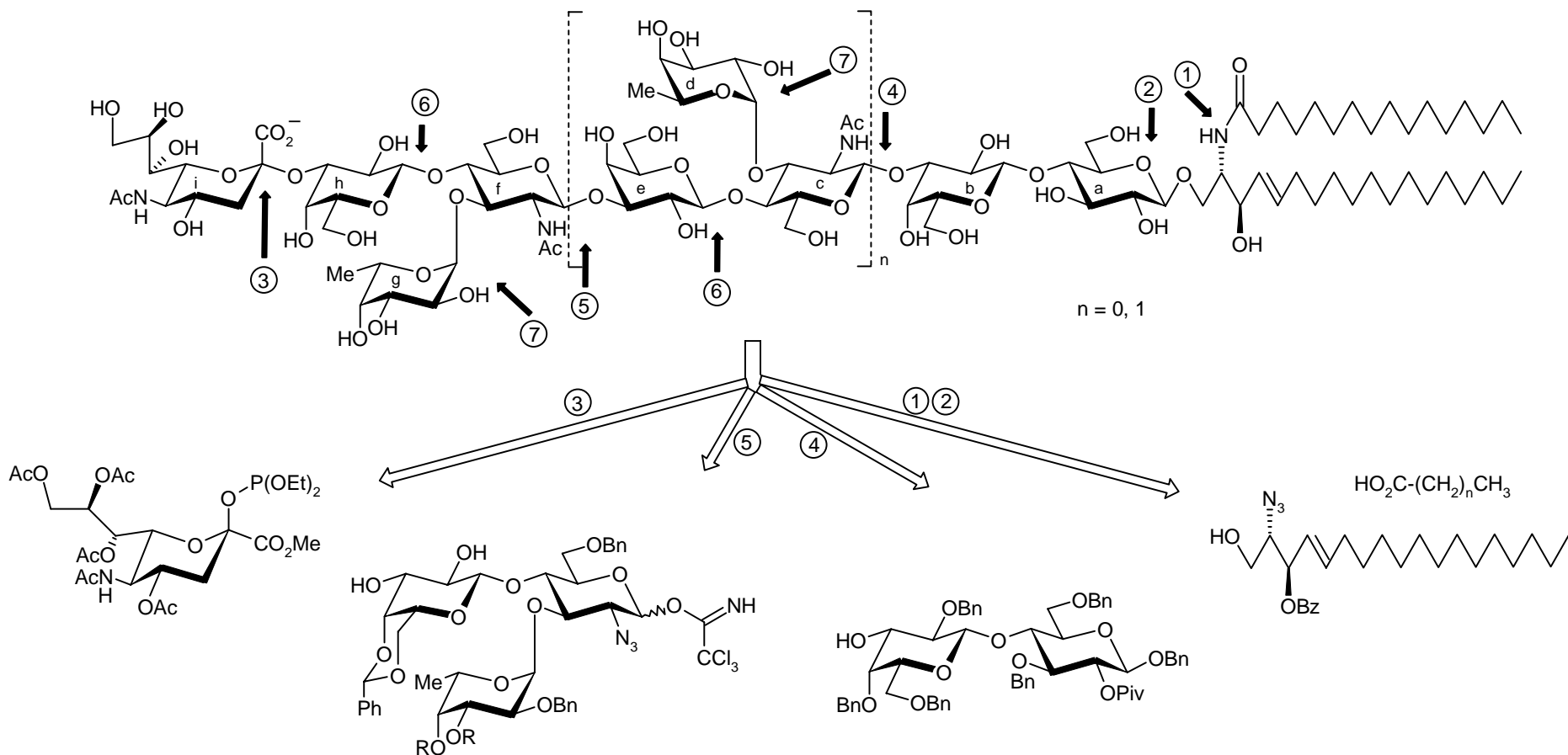
"While the functions of DNA and proteins are generally known, it is much less clear what carbohydrates do"?

"The roles of oligosaccharides ... appear to range from trivial to crucial ..."

GLYCOLIPIDS

SYNTHESIS OF THE MONOMERIC, DIMERIC, TRIMERIC, AND TETRAMERIC sLe^x ANTIGEN

RETROSYNTHETIC ANALYSIS



STRATEGIES AND METHODS DEVELOPED IN THE BLOCK SYNTHESIS

GLYCOSYLATION

- Trichloroacetimidate LGs (Cat.)
- Phosphite LGs (Cat.)
- Anomeric O-Alkylation
- Inverse Procedure (IP)

STEREOCONTROL

- Anchimeric Assistance
- Anomeric Effect
- Nitrile Effect

PROTECTION

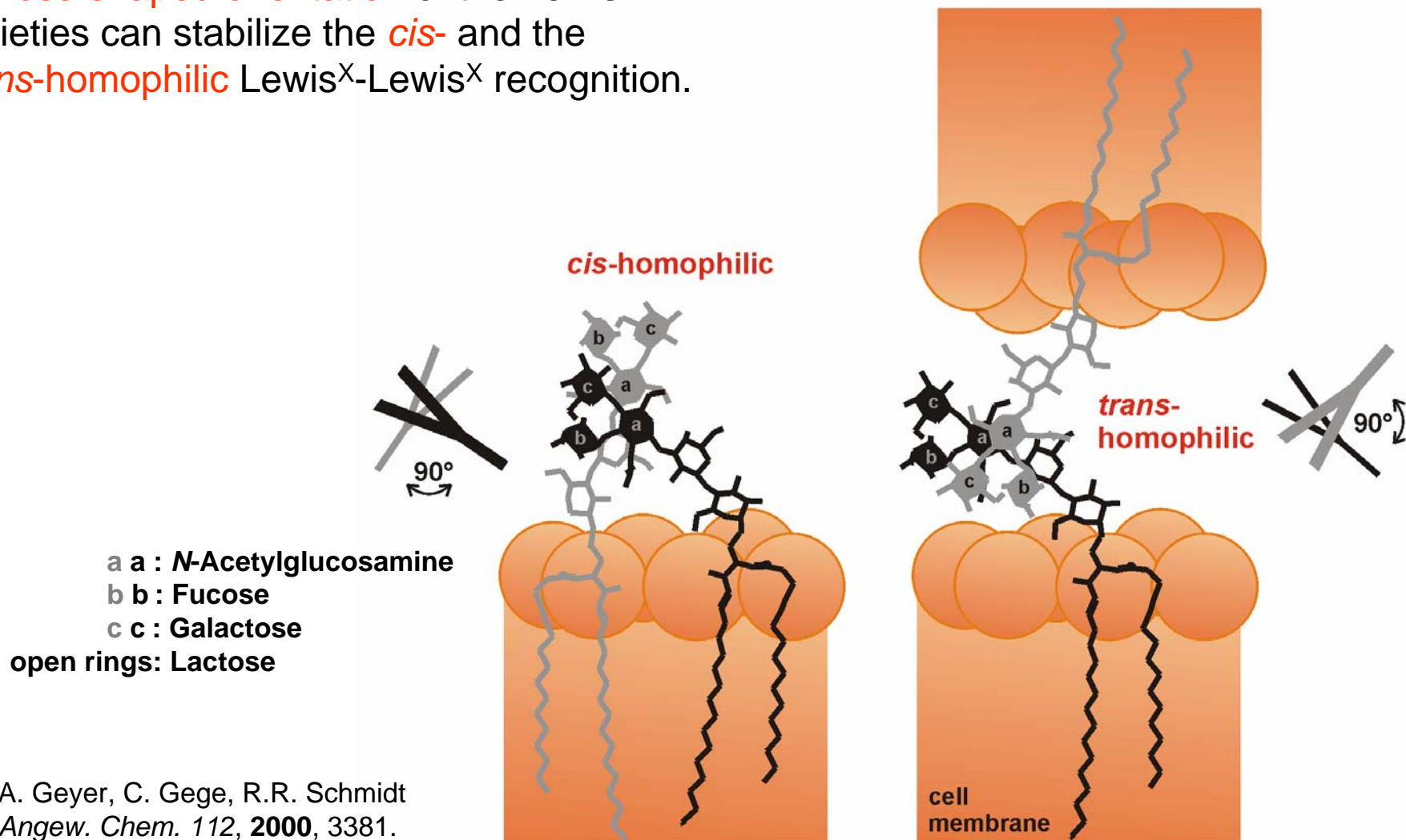
- OH: Bn, Ac, Bz, Piv
- NH₂: "N₂", TCP, DMM, Troc
- Diacyl, Thiodiacyl

CERAMIDE ATTACHMENT

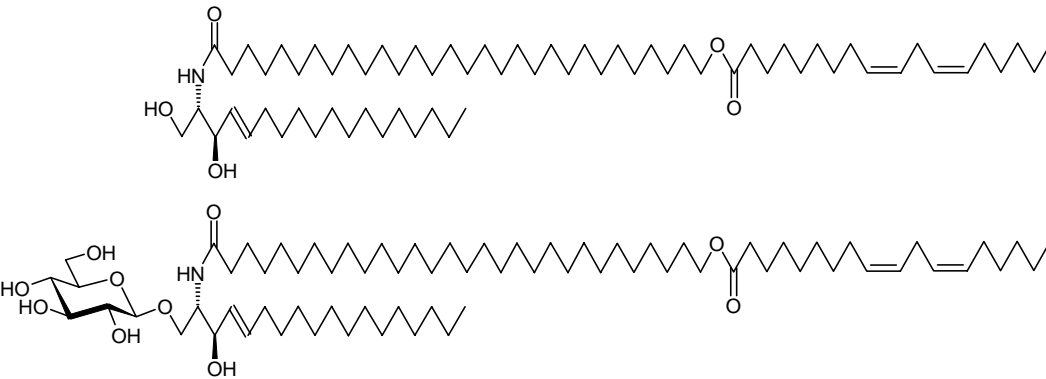
- Azidosphingosine Glycosylation
- Final glycosidic linkage with peracylated TCAI donor

Dimerisation of Lewis^X on the Cell Membrane – a Model

A **cross-shaped orientation** of the Lewis^X moieties can stabilize the **cis-** and the **trans-homophilic** Lewis^X-Lewis^X recognition.

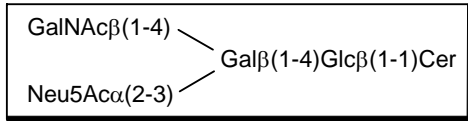


EPIDERMIS SPHINGOLIPIDS
CERAMIDES, CEREBROSIDES

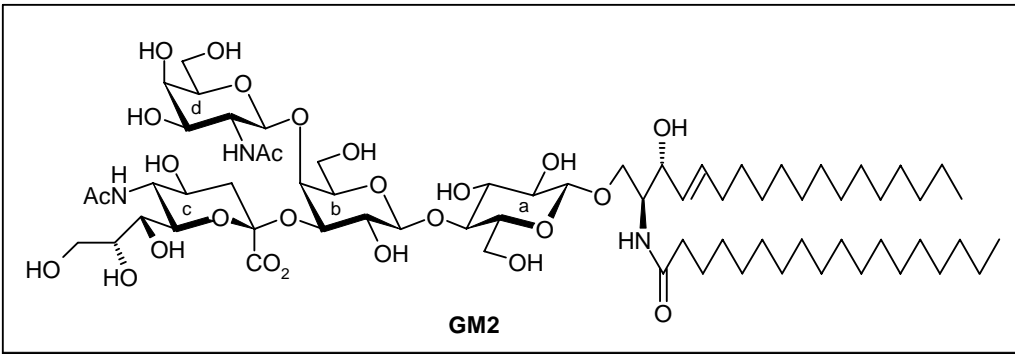


GANGLIOSIDES

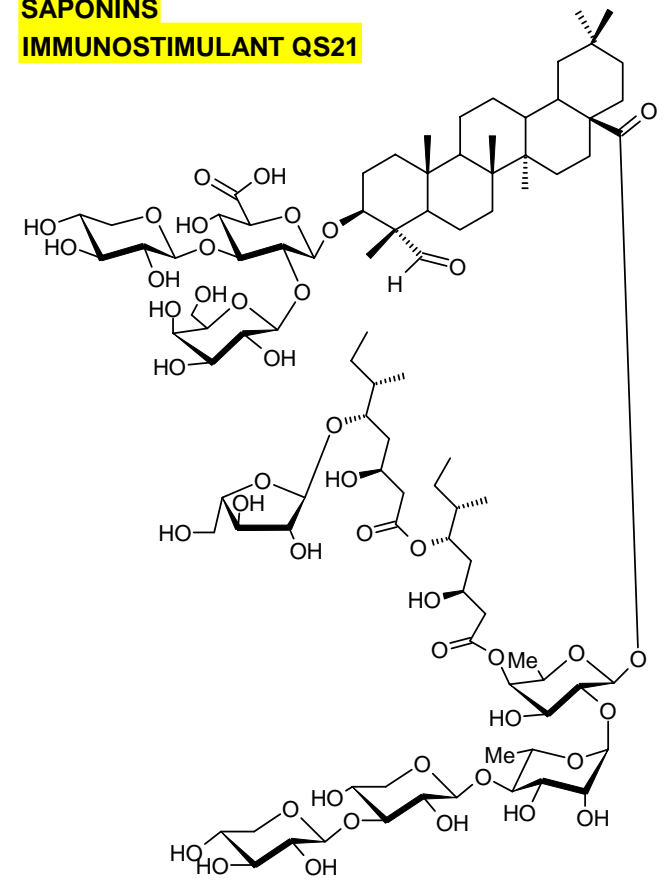
Efficient Synthesis of Ganglioside GM2 for Use in Cancer Vaccine Generation



|||

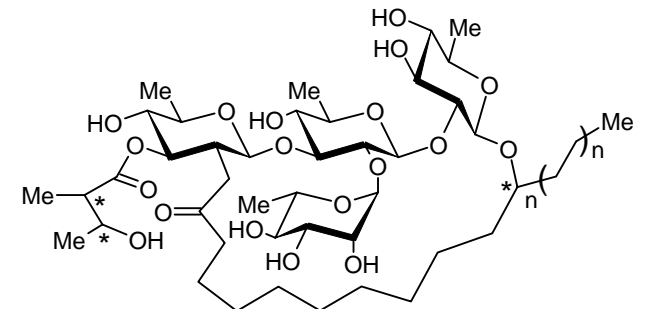


SAPONINS
IMMUNOSTIMULANT QS21

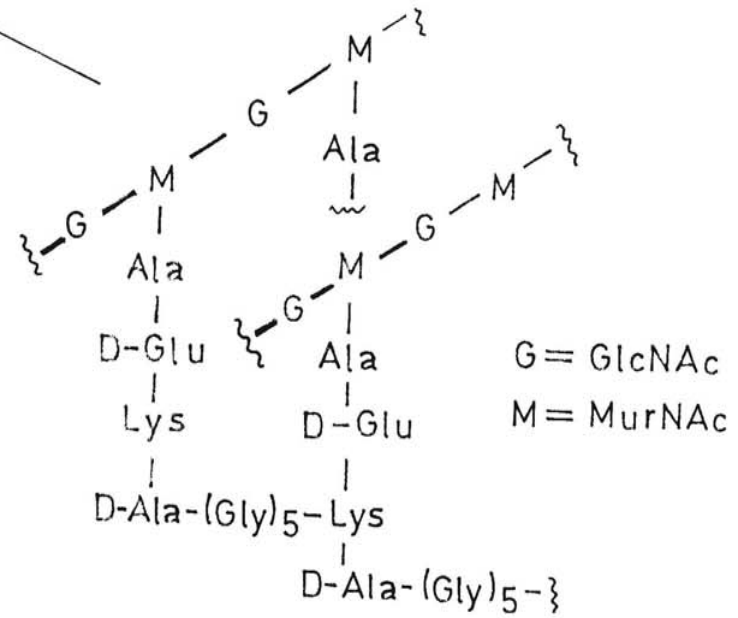
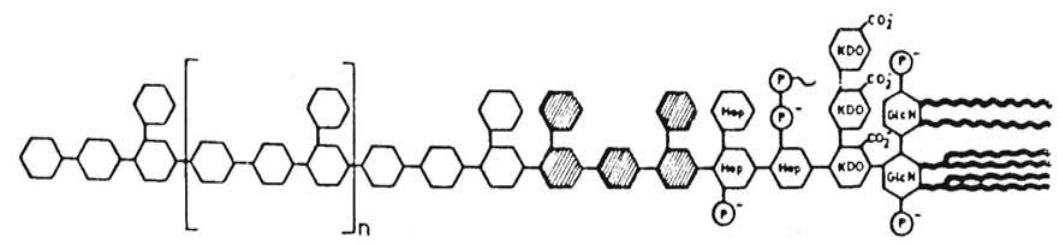
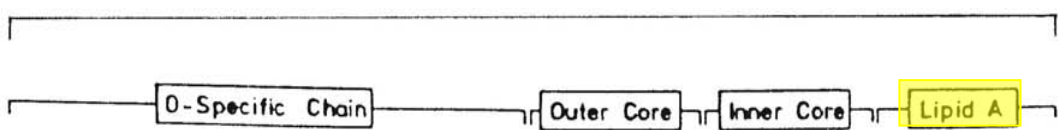
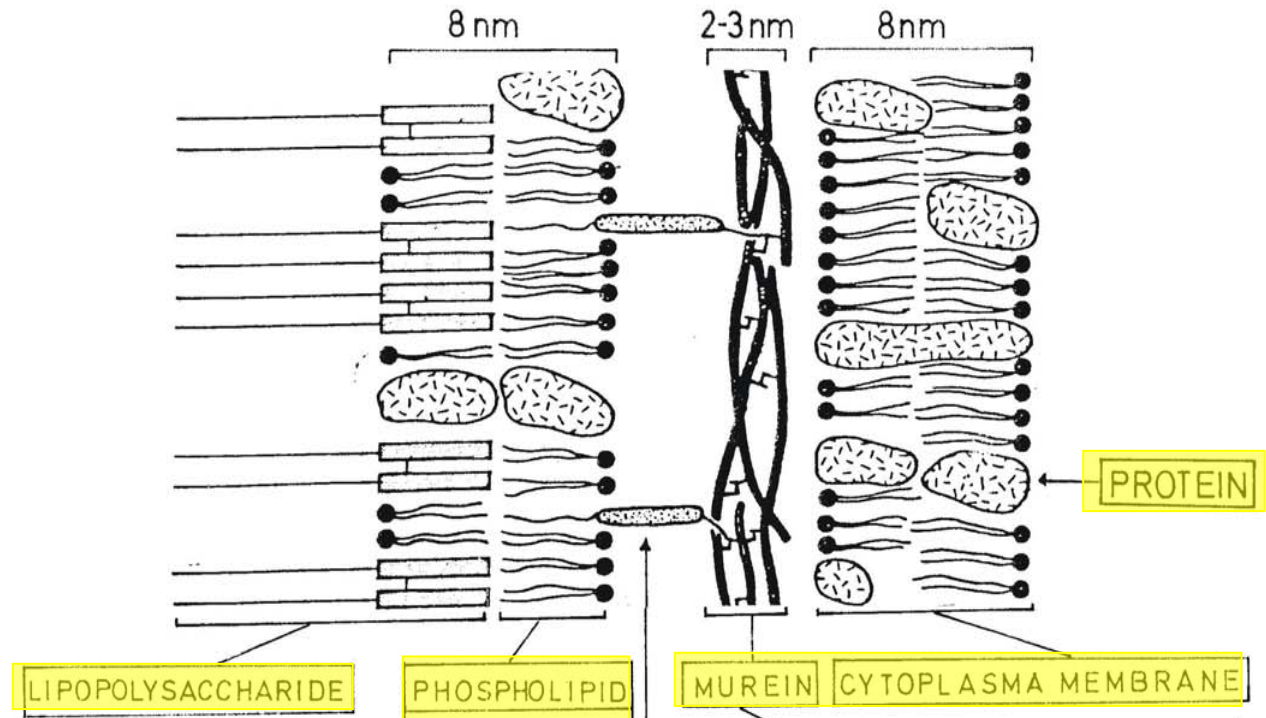


RESIN GLYCOSIDES

PLANT GROWTH REGULATOR CALONYCTIN A

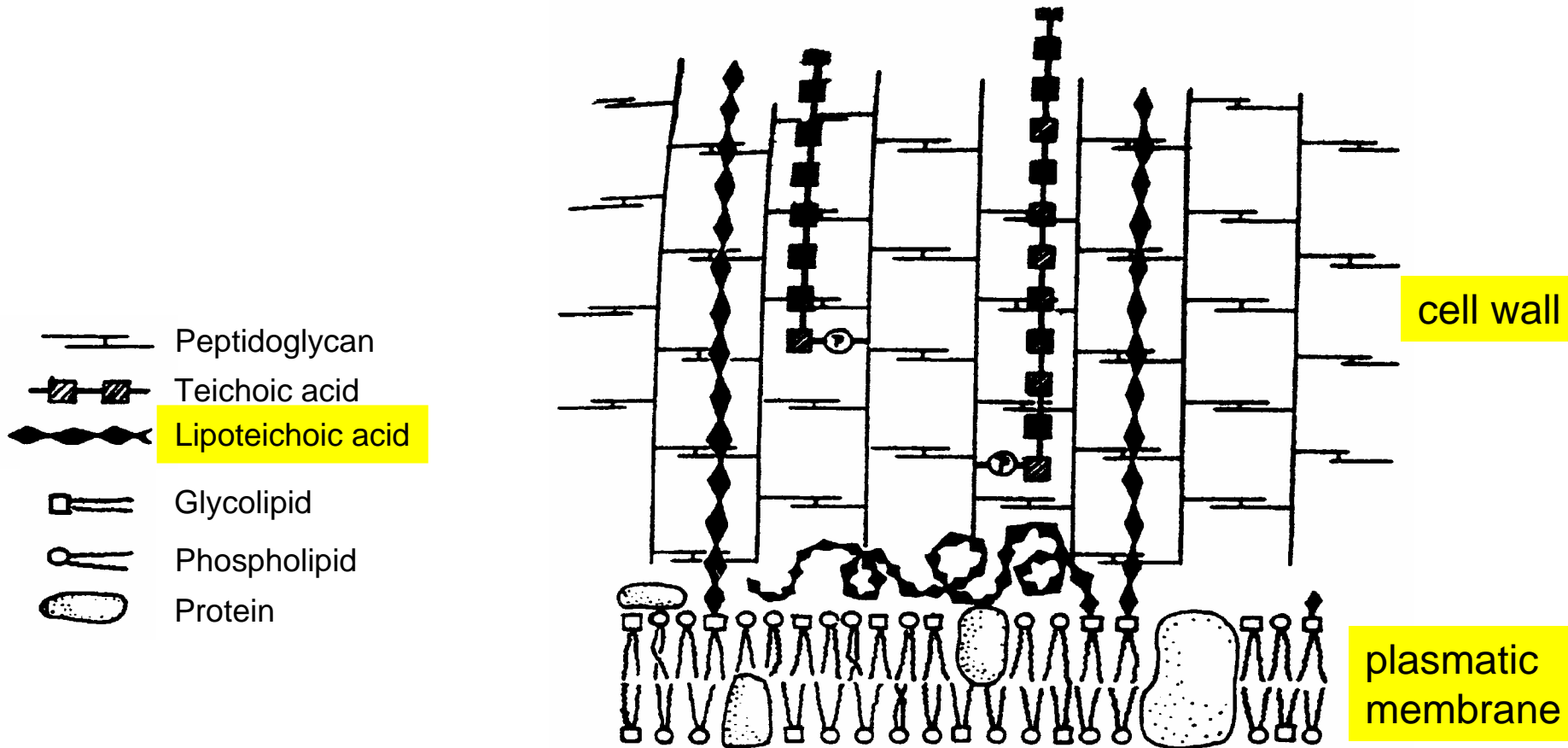


CELL WALL STRUCTURE OF GRAM NEGATIVE BACTERIA



CELL WALL-MEMBRANE COMPLEX OF GRAM-POSITIVE BACTERIA

MODEL



Lipoteichoic Acid (LTA) = Glycophospholipids

BIOLOGICAL ACTIVITY OF LTA – A SHORT OVERVIEW

The inflammatory response to Gram-negative and Gram-positive bacteria can hardly be distinguished. However, while most of the responses to Gram-negative bacteria could be attributed to lipopolysaccharides (LPS) and their lipid anchor lipid A, as general principles,^[1] no corresponding principle for Gram-positive bacteria was identified unambiguously during the last few decades.

Lipoteichoic acids (LTAs) are found in most Gram-positive bacteria. Like LPS, they are amphiphilic, negatively charged glycolipids. Yet, studies with commercial LTA preparations exhibiting significant activities could be traced back to contaminations with LPS. Furthermore, phenol-extracted LTA from different sources turned out to be essentially inactive and cytokine release as a measure of immunostimulatory activity was not observed.^[2]

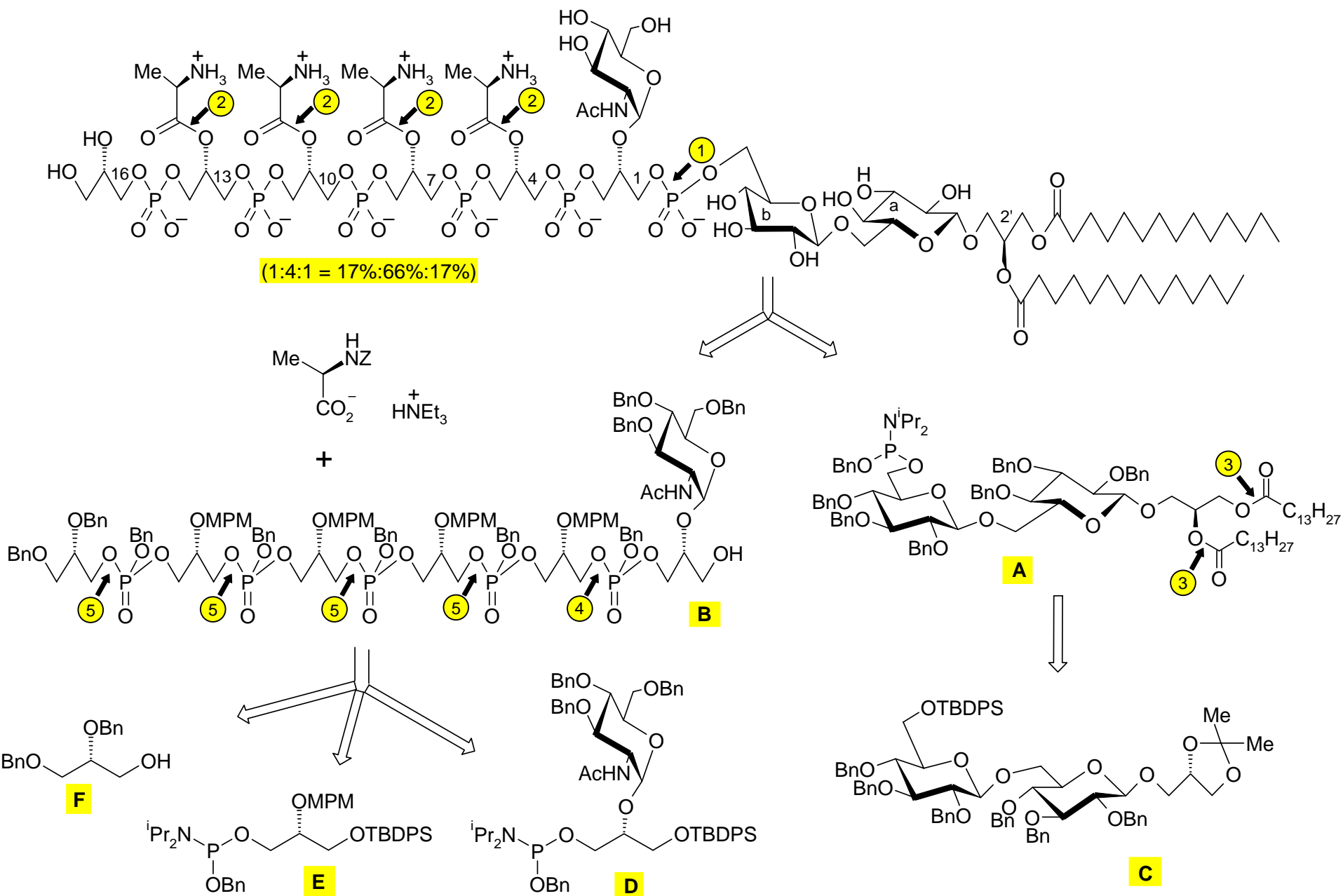
Only recently it was shown that these results are due to the preparation method employed for LTA: a less harsh preparation method led to pure, biologically active LTA.^[3] A crucial role could be attributed to preserved D-alanine residues at the polyglycerophosphate back-bone of LTA, for instance from *Staphylococcus aureus*.

[1] S. M. Opal, J. Cohen (1999)

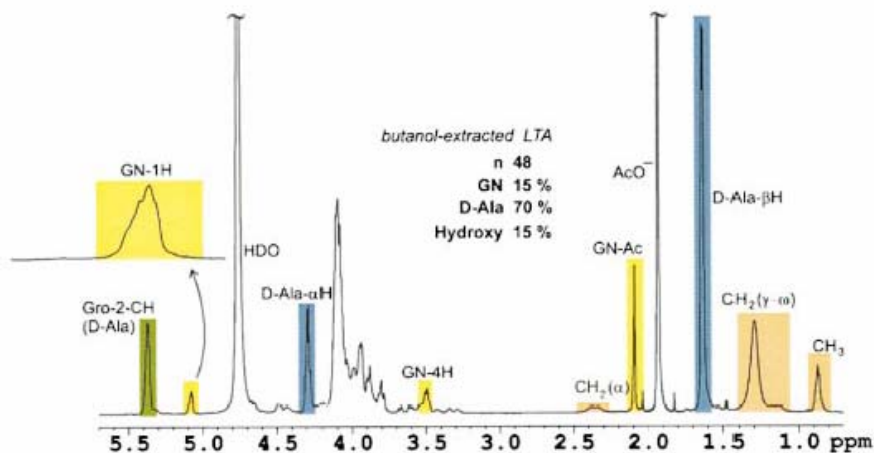
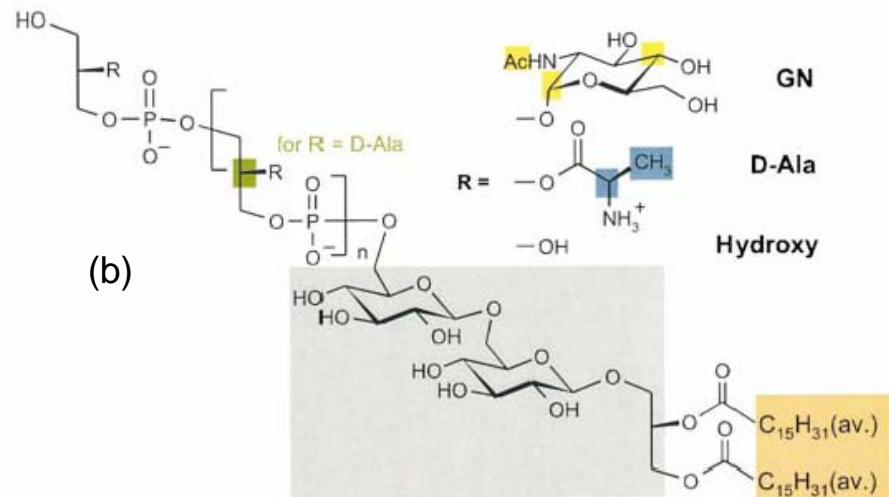
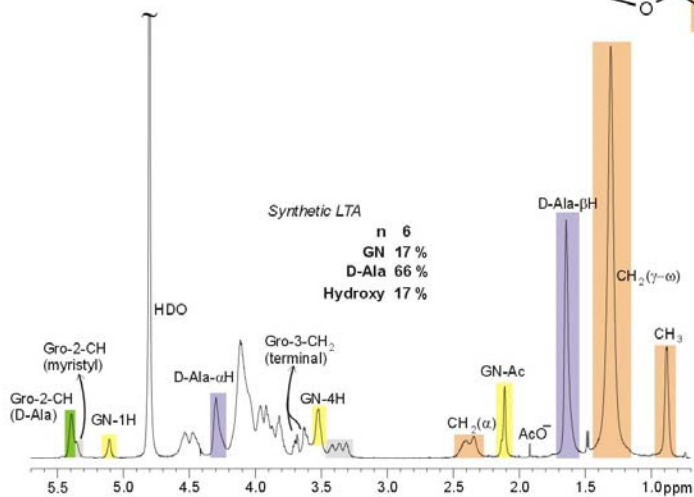
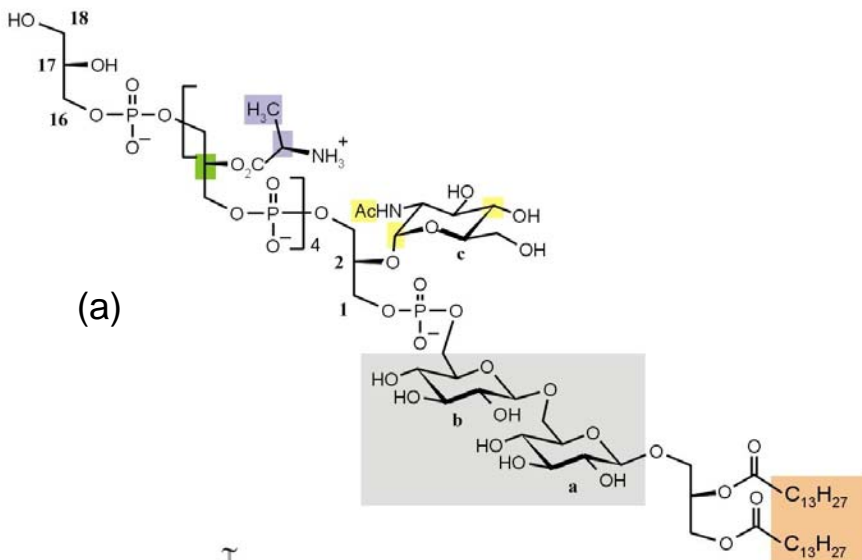
[2] T. Kusunoki, al. (1995)

[3] T. Hartung, al. (2001)

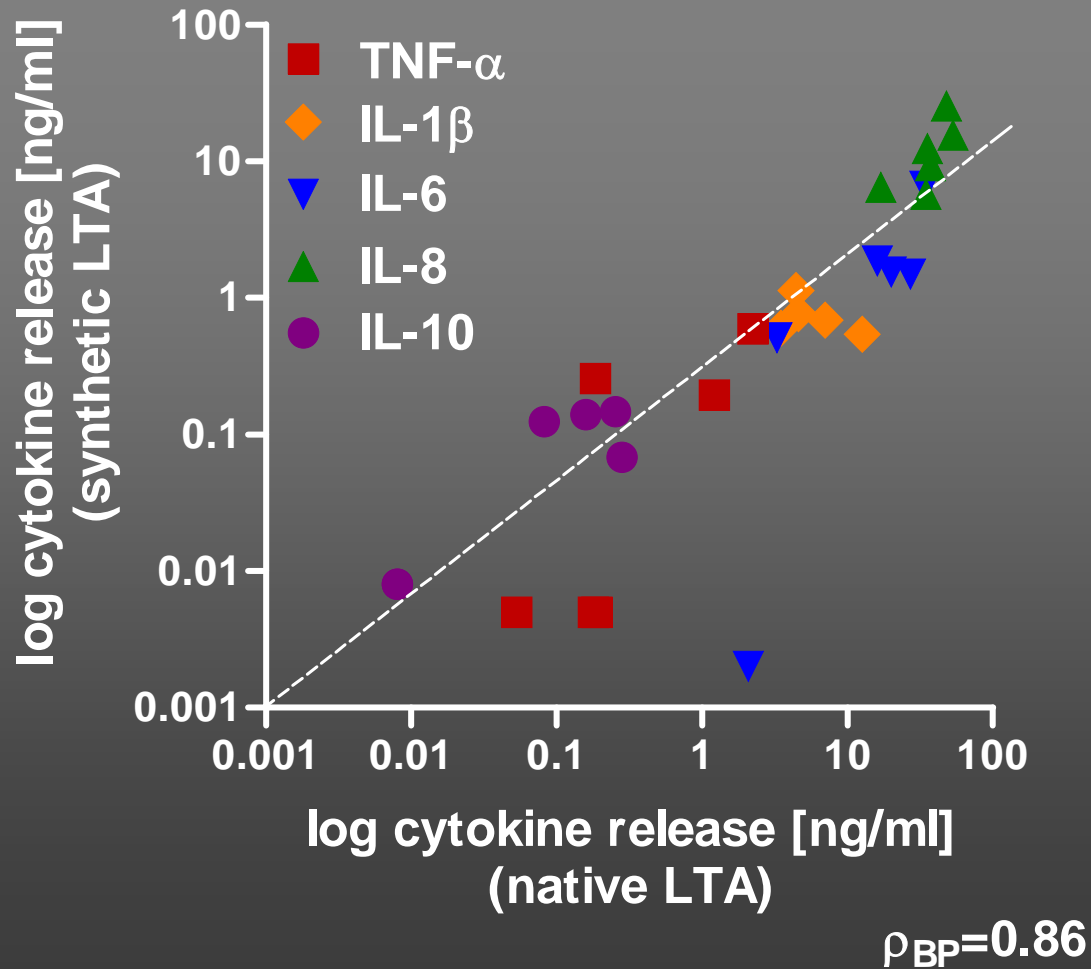
RETROSYNTHESIS OF A LIPOTEICHOIC ACID (LTA) OF *STAPHYLOCOCCUS AUREUS*



COMPARISON OF THE ¹H NMR SPECTRA OF SYNTHETIC (a) AND OF NATIVE LTA (b)



Synthetic and native LTA induce similar cytokine pattern

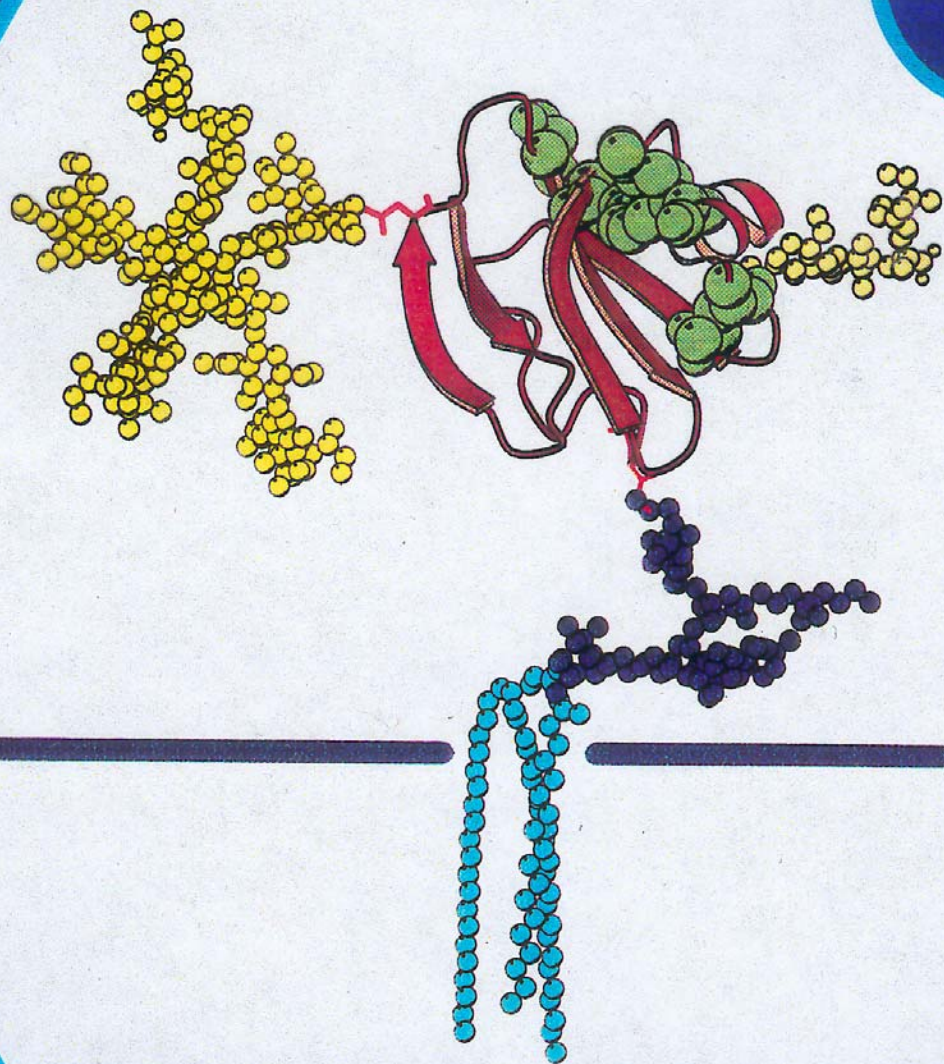


CONCLUSIONS

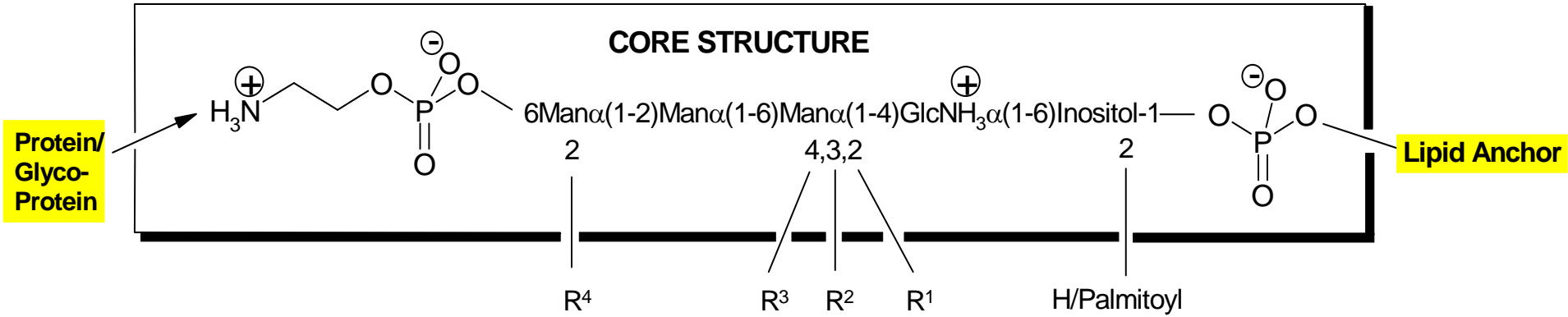
- Exchange of D-Alanine by L-Alanine reduces immunostimulatory potency
 - Stereoselective Recognition
- Deletion of GlcNAc or the Gentiobiose residue had no effect on immunostimulatory potency
 - Key Constituents: LTA Anchor
 - Two Fatty Acids
 - Glycerophosphate Backbone
 - D-Alanine Substituents

**GLYCOPHOSPHOLIPIDS
AND**

GLYCOPEPTIDES/GLYCOPROTEINS



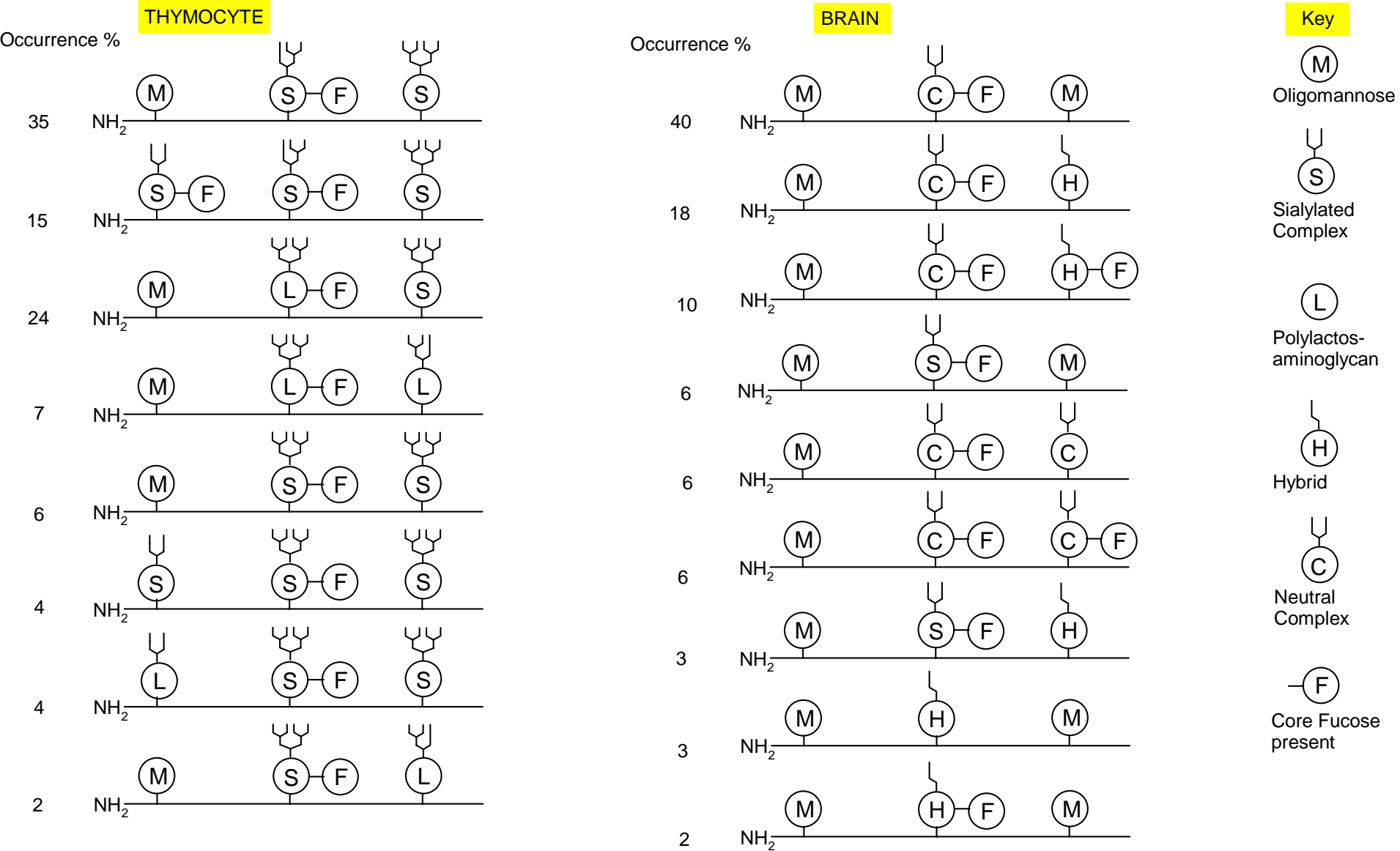
GENERAL STRUCTURE OF GPI ANCHORS



Natural Source	R^4	R^3	R^2	R^1	Lipid
<i>S. cerevisiae</i>	$\text{Man}\alpha(1-2)$	H	H	H	Ceramide/DAG
<i>T. brucei</i> VSG	H	H	$\text{Gal}_{2-4}\alpha(1-3)$	H	DAG
<i>T. gondii</i> A	H	$\text{GalNAC}\beta(1-4)$	H	H	DAG
<i>T. gondii</i> B	H	$\text{Glc}\alpha(1-4)\text{GalNAC}\beta(1-4)$	H	H	DAG
Rat brain <i>Thy-1</i>	$\text{Man}\alpha(1-2)$	$\text{GalNAC}\beta(1-4)$	H	EA-P	Acyl alkyl glycerol

GLYCOFORMS OF THYMOCYTE AND BRAIN-DERIVED RAT THY-1 GLYCOPROTEIN

(R. A. Dwek, 1996)



NUMBER OF DIFFERENT CONNECTIONS BETWEEN AMINO ACIDS, NUCLEOTIDES, AND SUGARS, RESP.^a

Number of constituents	NUMBER OF DIFFERENT CONNECTIONS		
	Peptides/Nucleic Acids	Saccharides	
$1 \times Z$	1	Unbranched^b 1	
$2 \times Z$	1	11	
$3 \times Z$	1	120	
$4 \times Z$	1	1.424	
$5 \times Z$	1	17.872	
Z	1	Unbranched^c 2	Branched^c 2
Y + Z	2	128	256
X + Y + Z	6	6.144	38.016
W + X + Y + Z	24	393.216	7.602.176
V + W + X + Y + Z	120	31.457.280	~ 2.633.600.000
U + V + W + X + Y + Z	720	3.019.898.880	~ 1.053.045.031.000

^a Calculations of A. Enhsen and R. R. Schmidt, Univ. Konstanz, **1986**
R. A. Lane, *Glycobiology*, **1994**, 4, 759-767

^b Hexopyranoses

^c Hexopyranoses + Hexofuranoses

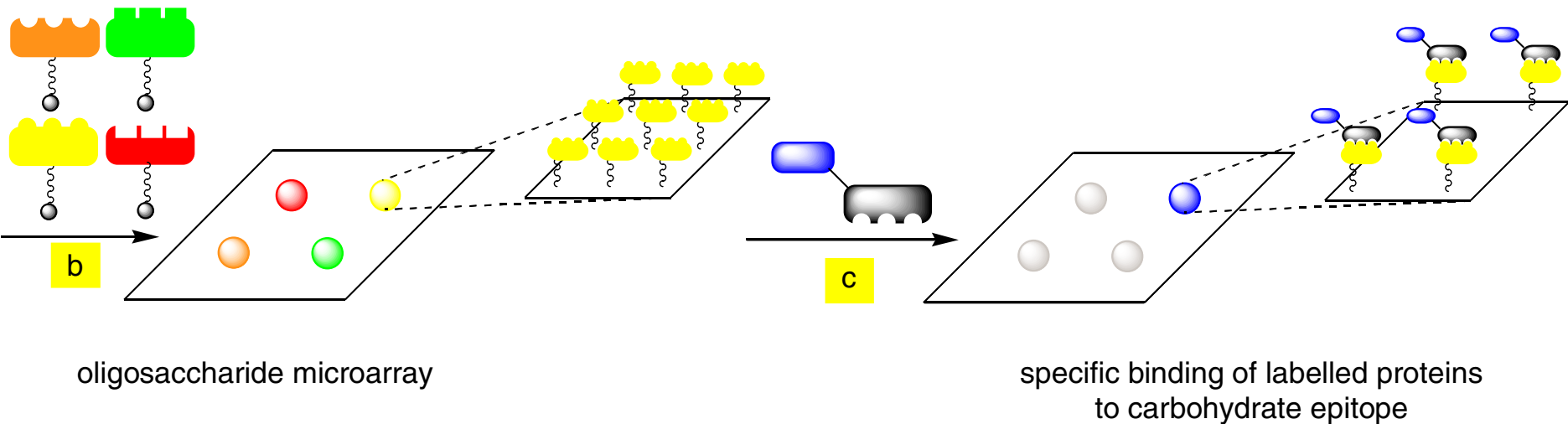
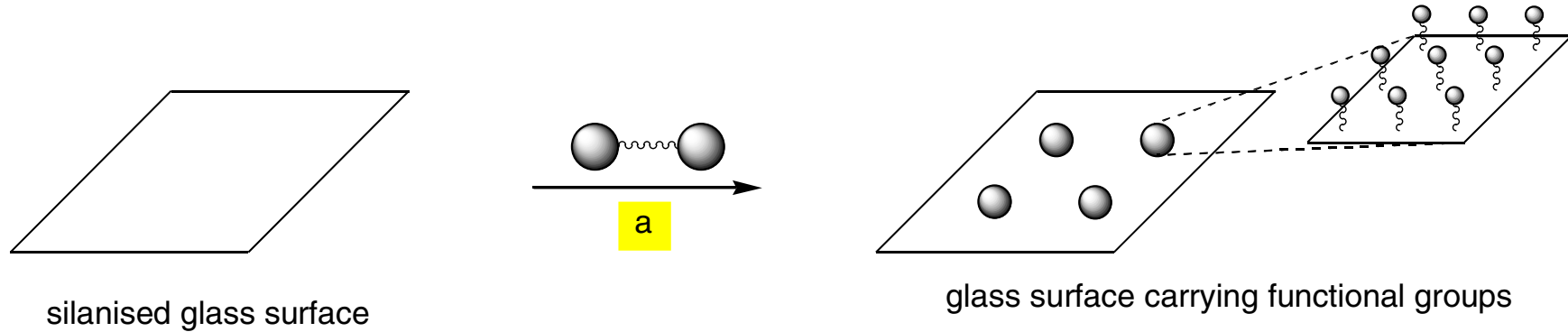
GENOME, PROTEOME, GLYCOME

All Genes (static figure)	=	GENOME
From the Genome Expressed Proteins (dynamic figure)	=	PROTEOME
From the Genome Expressed Glycans (via posttranslational modification) (dynamic figure)	=	"GLYCOME"

ANALYTICAL TOOLS

CARBOHYDRATE CHIPS FOR PROBING CARBOHYDRATE-PROTEIN AND CARBOHYDRATE-CARBOHYDRATE INTERACTIONS

PROCESS FOR DETECTING CARBOHYDRATE-PROTEIN INTERACTION VIA MICROARRAY TECHNIQUES



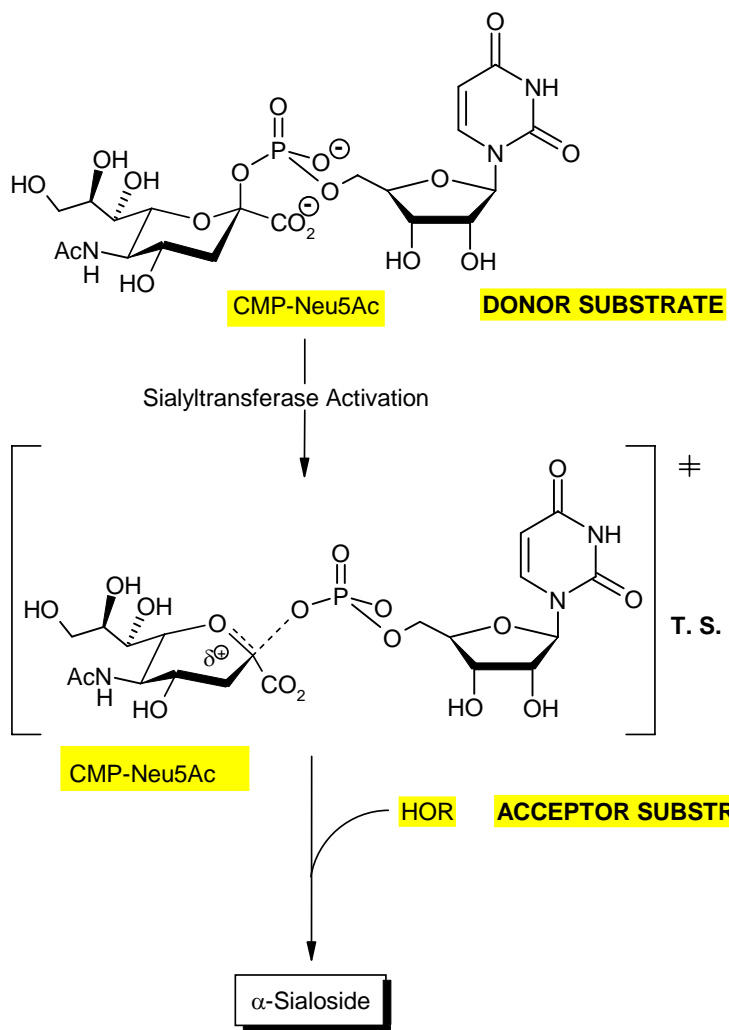
- a) derivatisation of glass surface with suitable functional groups
- b) coupling of oligosaccharides to different spots
- c) hybridisation with labelled protein

CONTROL OF

- GLYCOSIDE BOND FORMATION BY
GLYCOSYLTRANSFERASES,
TRANSGLYCOSIDASES:
SELECTIVE INHIBITORS**

- GLYCOSIDE BOND DEGRADATION BY
GLYCOSIDASES:
GLYCOSIDASE INHIBITORS**

Sialyl Transfer: S_N1-Type Mechanism



R. R. Schmidt, al. 1996
B. A. Horenstein, al. 1996

Transition State Analogues

- (1) Planar Anomeric Carbon (sp²)
- (2) Extension of Distance to CMP Leaving Group
- (3) Two Negative Charges; Distance 5 or 6 Bonds
- (4) Replacement of Neuraminy Residue by Aryl, Hetaryl, etc.
- (5) Different Geometry of CMP Leaving Group and Carboxylate Equivalent at Anomeric Carbon

	K _M [μmol]	K _i [μmol]	K _M /K _i
CMP-Neu5Ac	46	-	-
	-	h 0.029 ± 0.006	1580
	-	I 0.69 ± 0.19	67
	-	h 0.059 ± 0.018	780
	-	I 0.038 ± 0.009	1210
	-	E 158 ± 41*	0.3
	-	Z 25 ± 7	1.8
	-	E 2.4 ± 0.4	19
	-	Z 3.5 ± 1.4	13
* Because of low solubility this compound was dissolved in DMSO and the mixture diluted with buffer.			
**Inhibition mode: competitive			

**SYNTHESIS OF
STRUCTURALLY DEFINED
GLYCOPEPTIDES/GLYCOPROTEINS**

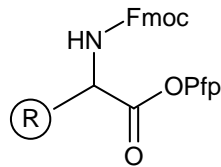
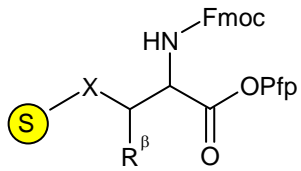
GLYCOPEPTIDE/GLYCOPROTEIN SYNTHESIS: GENERAL ASPECTS

in vitro

Amino Acids
(20 Proteinogenic AA's)

Amino Group Protection
Carboxylate Group Activation

Synthesis of O-/N-Glycosyl Amino Acids



(R) = Side Chain

(S) = Sugar

X = O, R^β = H, Me (**Ser/Thr**) — O-GLYCANS
X = CONH, R^β = H (**Asn**) — N-GLYCANS

Sequence Specific
Chain Extension

Linear Solution/
Solid Phase
Synthesis

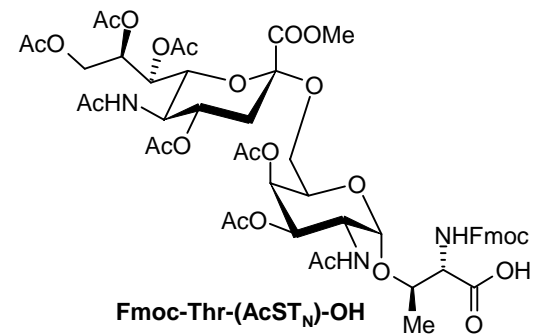
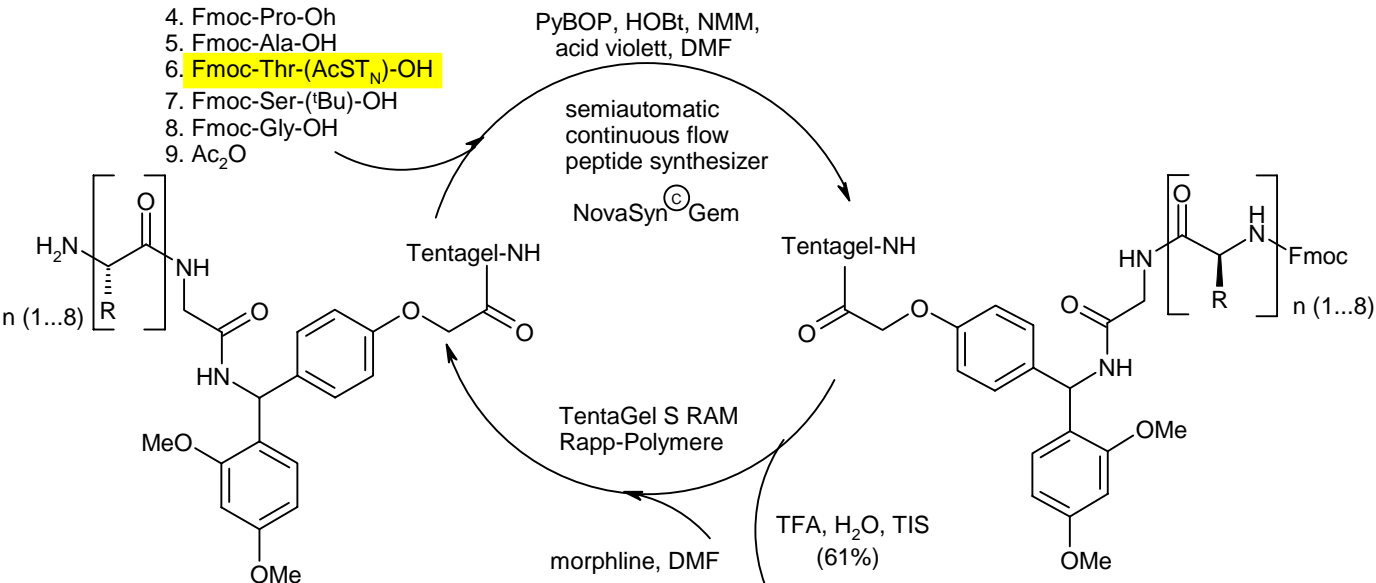
Deprotection

Mild Base and/or
Acid Treatment

Glycopeptide

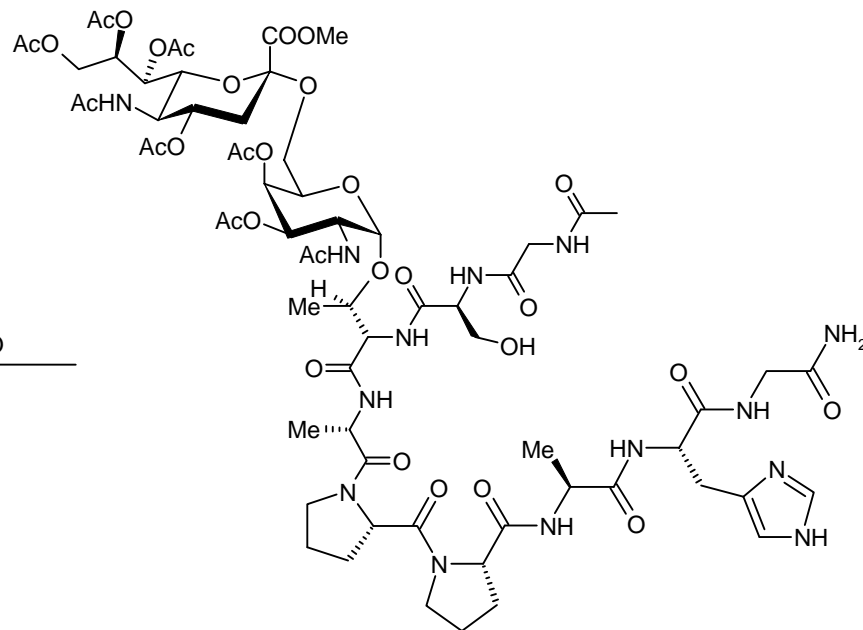
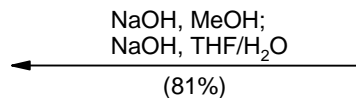
SYNTHESIS OF A GLYCOPEPTIDE BEARING THE ST_N ANTIGEN

1. Fmoc-His-(Trt)-OH
2. Fmoc-Ala-OH
3. Fmoc-Pro-OH
4. Fmoc-Pro-OH
5. Fmoc-Ala-OH
6. **Fmoc-Thr-(AcST_N)-OH**
7. Fmoc-Ser-(^tBu)-OH
8. Fmoc-Gly-OH
9. Ac₂O

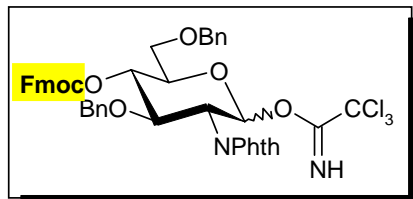
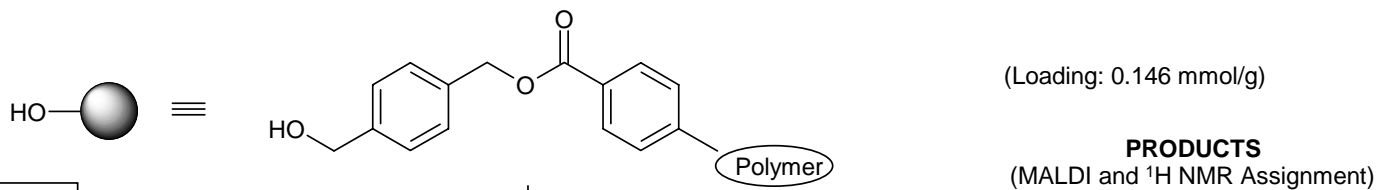


Neu5Ac α (2-6)GalNAc α (1-0)

Ac-GSTAPPAHG-NH₂

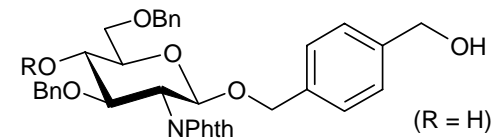


SYNTHESIS OF THE BRANCHED CORE STRUCTURE OF N-GLYCANS

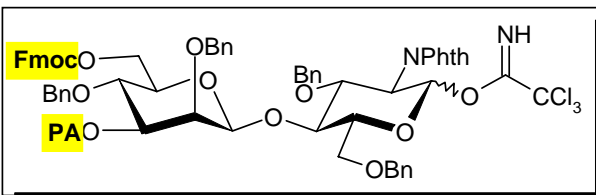
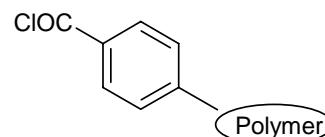


TMSOTf, CH₂Cl₂, -40 °C

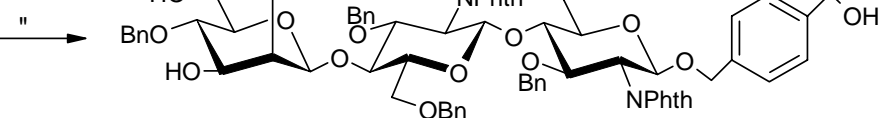
NaOMe (4 eq)
CH₂Cl₂/MeOH (8:1)



NEt₃/CH₂Cl₂
(1:8)

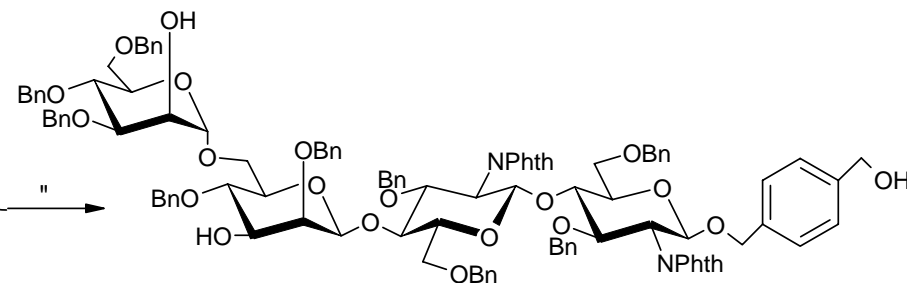


TMSOTf, CH₂Cl₂, -20 °C



NEt₃/CH₂Cl₂
(1:8)

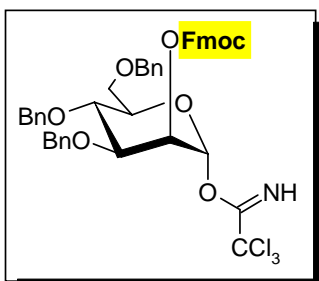
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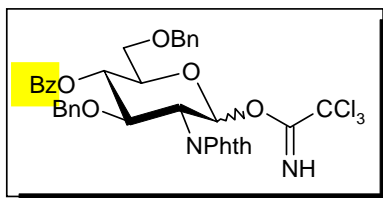
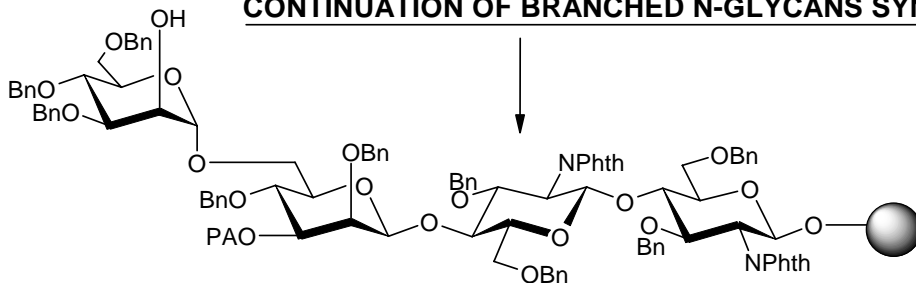
TMSOTf, CH₂Cl₂/Et₂O (4:1)
-20 °C

NEt₃/CH₂Cl₂
(1:8)

"



CONTINUATION OF BRANCHED N-GLYCANS SYNTHESIS



TMSOTf, CH₂Cl₂/MeCN (4:1)
-40 °C

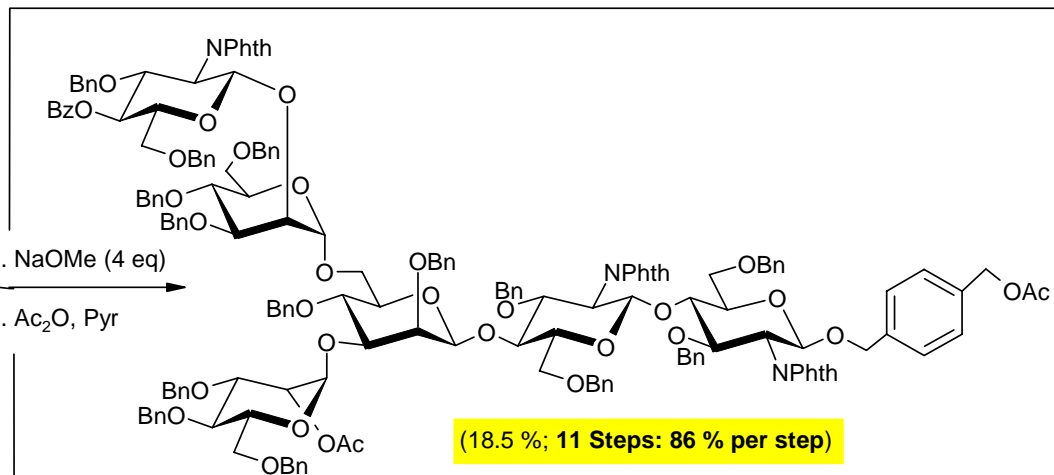
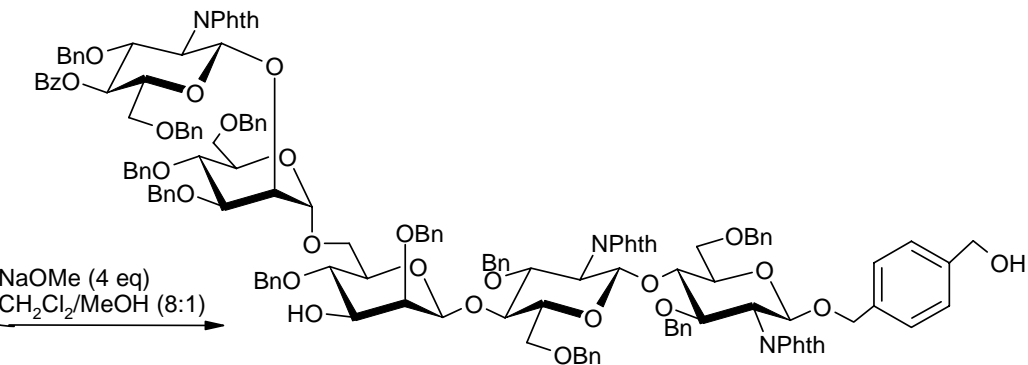
NaOMe (0.5 eq)
CH₂Cl₂/MeOH (1:8)

NaOMe (4 eq)
CH₂Cl₂/MeOH (8:1)

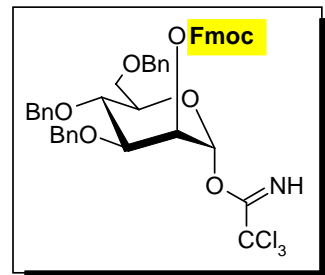
TMSOTf, CH₂Cl₂/Et₂O (4:1)
-20 °C

1. NaOMe (4 eq)

2. Ac₂O, Pyr



(18.5 %; 11 Steps: 86 % per step)



GLYCOPEPTIDE/GLYCOPROTEIN SYNTHESIS: GENERAL ASPECTS

in vitro

in vivo

Amino Acids
(20 Proteinogenic AA's)

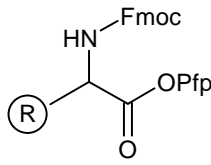
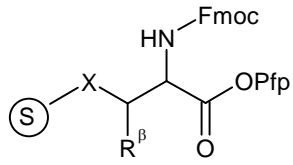
Amino Group Protection
Carboxylate Group Activation

Carboxylate Activation
Attachment to AA-specific tRNA's

Synthesis of O-/N-Glycosyl Amino Acids

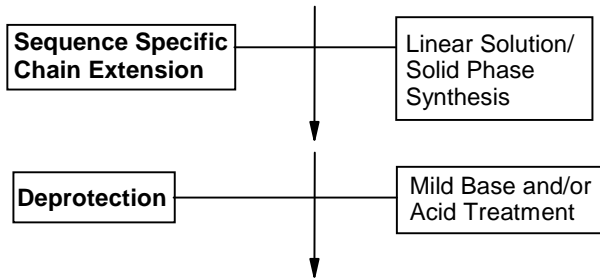
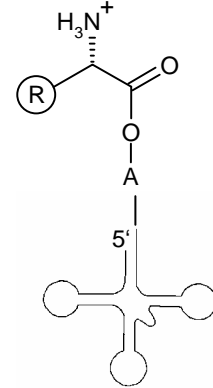
ATP, tRNA-Synthetase
tRNA's

(R) = Side Chain

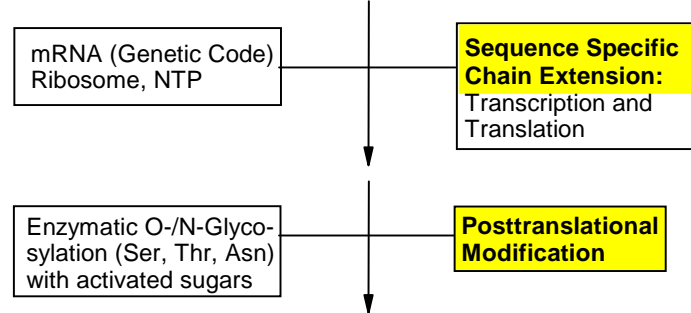


(S) = Sugar

X = O, R^β = H, Me (**Ser/Thr**)
X = CONH, R^β = H (**Asn**)



Glycopeptide



Glycopeptide/Glycoprotein

GLYCOPEPTIDE/GLYCOPROTEIN SYNTHESIS: GENERAL ASPECTS

in vitro

in vivo

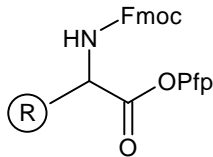
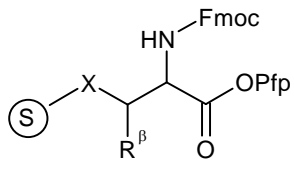
Amino Acids
(20 Proteinogenic AA's)

Amino Group Protection
Carboxylate Group Activation

Carboxylate Activation
Attachment to AA-specific tRNA's

ATP, tRNA-Synthetase
tRNA's

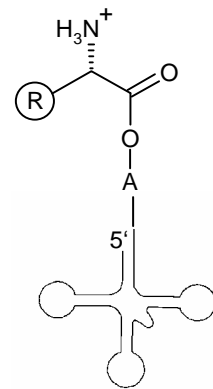
Synthesis of O-/N-Glycosyl Amino Acids



(R) = Side Chain

(S) = Sugar

X = O, R^β = H, Me (**Ser/Thr**)
X = CONH, R^β = H (**Asn**)



"CHEMICAL LIGATION"
OF PEPTIDE WITH
GLYCOPEPTIDE BLOCKS

Sequence Specific
Chain Extension

Linear Solution/
Solid Phase
Synthesis

mRNA (Genetic Code)
Ribosome, NTP

Sequence Specific
Chain Extension:
Transcription and
Translation

Deprotection

Mild Base and/or
Acid Treatment

Enzymatic O-/N-Glyco-
sylation (Ser, Thr, Asn)
with activated sugars

Posttranslational
Modification

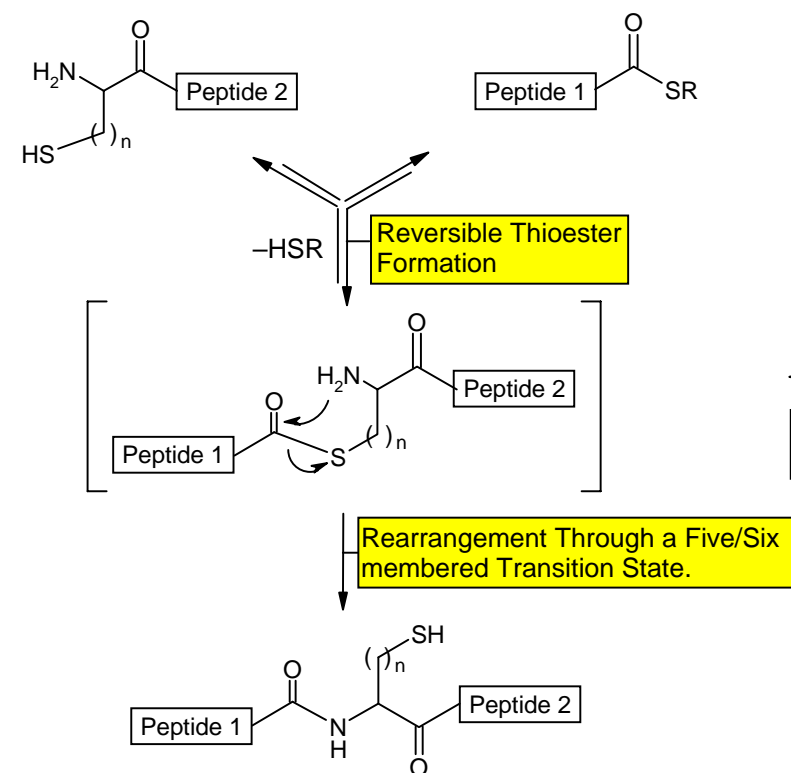
Glycopeptide

Glycopeptide/Glycoprotein

NATIVE CHEMICAL LIGATION

NATURE'S PROCEDURE (n = 1)

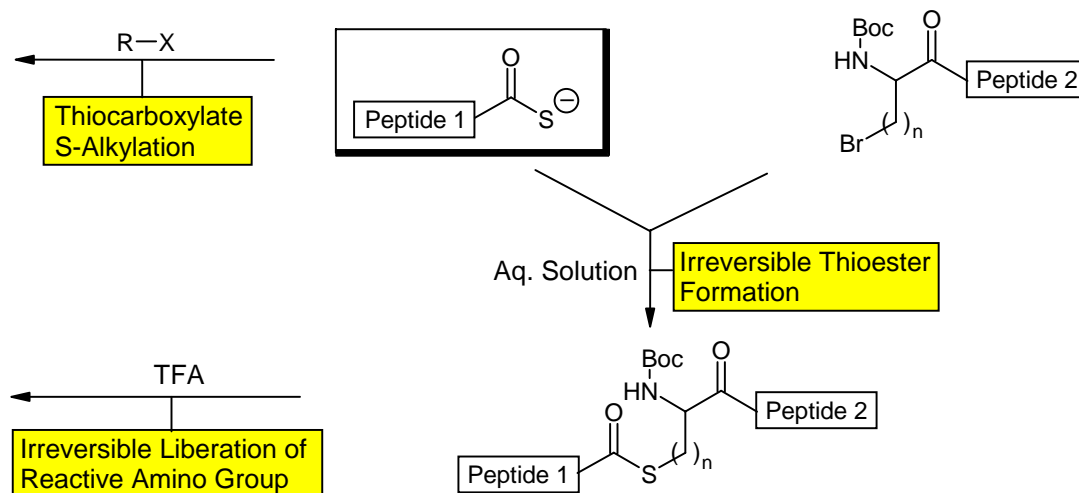
- (1) FORMATION OF A REACTIVE THIOESTER FROM THIOCARBOXYLATE
- (2) REVERSIBLE FORMATION OF A REACTIVE INTERMEDIATE



T. Wieland, al. 1953 (n = 1)
 S. B. H. Kent, al. 1994 (n = 1)
 J. P. Tam, al. 1998 (n = 2)
 K. Pachamuthu, R. R. Schmidt, 2003 (n = 2)

AN ALTERNATIVE PROCEDURE

- (1) IRREVERSIBLE FORMATION OF A STABLE INTERMEDIATE
- (2) LIBERATION OF THE REACTIVE INTERMEDIATE IN A SECOND STEP

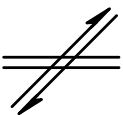
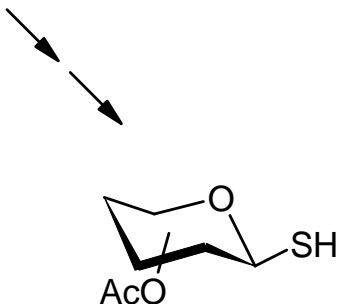
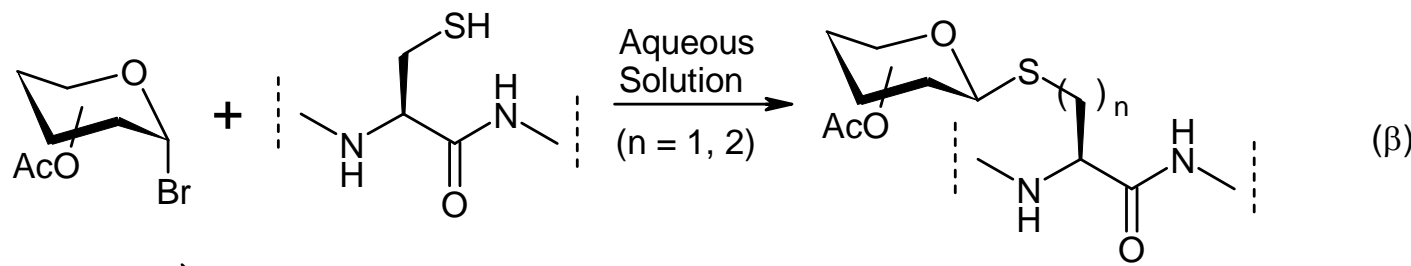


POSSIBLE ADVANTAGES

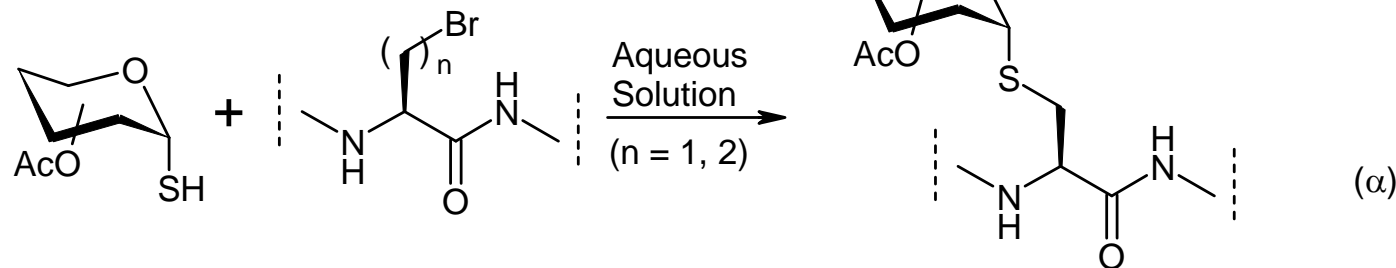
- (1) Convenient preparation of starting material.
- (2) Formation of hydrolytically quite stable thiothioesters.
- (3) Chemoselective highyielding formation of thioester intermediates which can be isolated and purified.
- (4) Convenient liberation of the reactive intermediate yielding essentially quantitative ligation.
- (5) Compatible with most amino acids.

STRATEGIES FOR THE SYNTHESIS OF α -S-LINKED AND OF β -S-LINKED GLYCOPEPTIDES

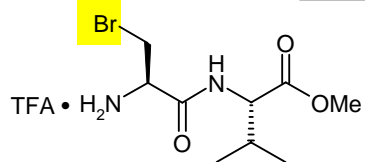
S-GLYCOSYLATION



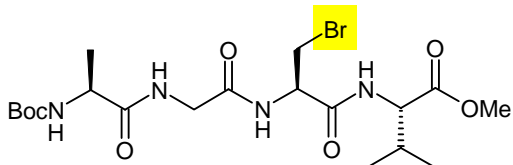
ANOMERIC S-ALKYLATION



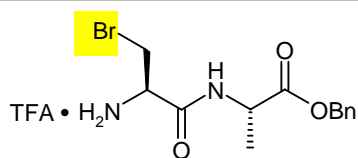
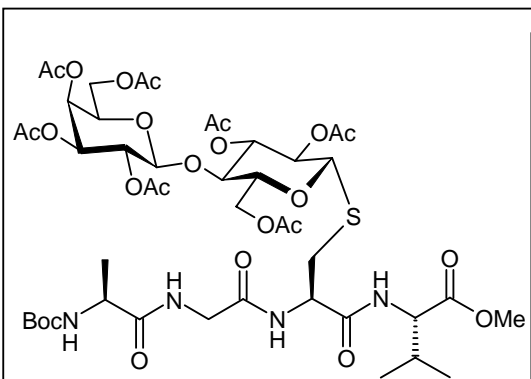
β -S-LINKED GLYCOPEPTIDES VIA ANOMERIC S-ALKYLATION OF β -LACTOSYLTHIOL



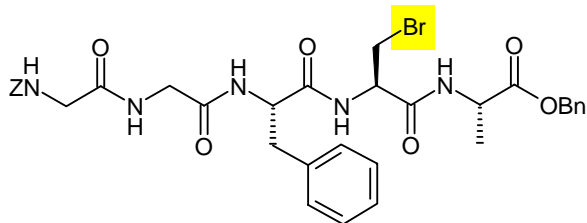
BocAlaGlyOH
PyBOP, DIPEA
(89%)



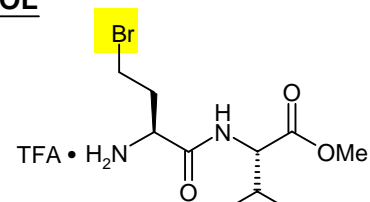
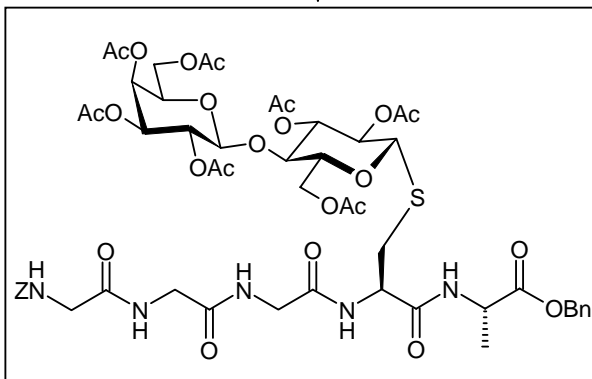
NaHCO₃, DMF/H₂O
(95%)



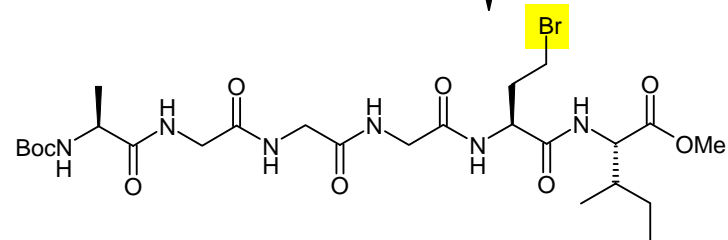
Z-Gly-Gly-Phe-OH
PyBOP, DIPEA
(83%)



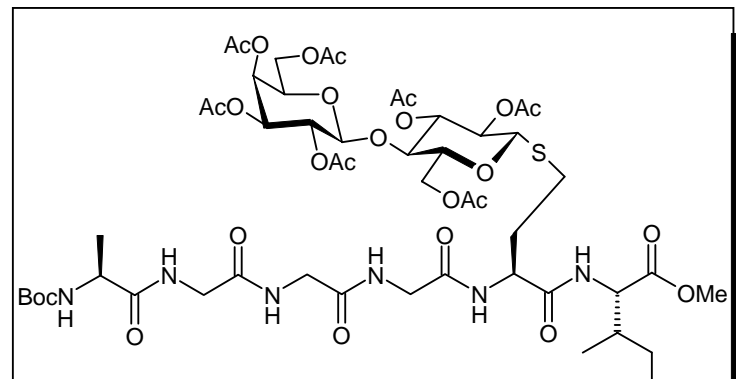
(94%)



Boc-Ala-Gly-Gly-Gly-OH
PyBOP, DIPEA
(70%)



(76%)
NaHCO₃, DMF/H₂O



GLYCOPEPTIDE/GLYCOPROTEIN SYNTHESIS: GENERAL ASPECTS

in vitro

in vivo

Amino Acids
(20 Proteinogenic AA's)

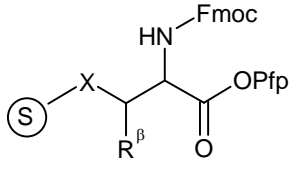
**Amino Group Protection
Carboxylate Group Activation**

Carboxylate Activation
Attachment to AA-specific tRNA's

**Synthesis of O-/N-
Glycosyl Amino Acids**

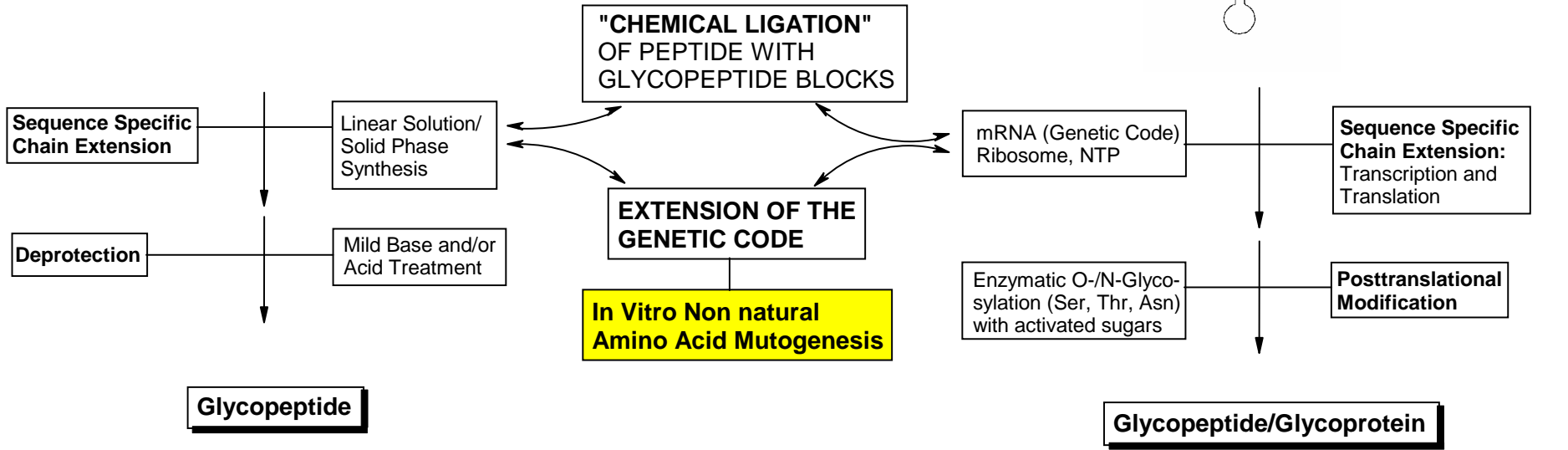
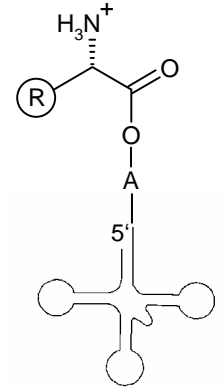
ATP, tRNA-
Synthetase
tRNA's

(R) = Side Chain



(S) = Sugar

X = O, R^β = H, Me (Ser/Thr)
X = CONH, R^β = H (Asn)



REGIOSPECIFIC INCORPORATION OF GLYCOSYLATED AMINOACIDS INTO PROTEINS

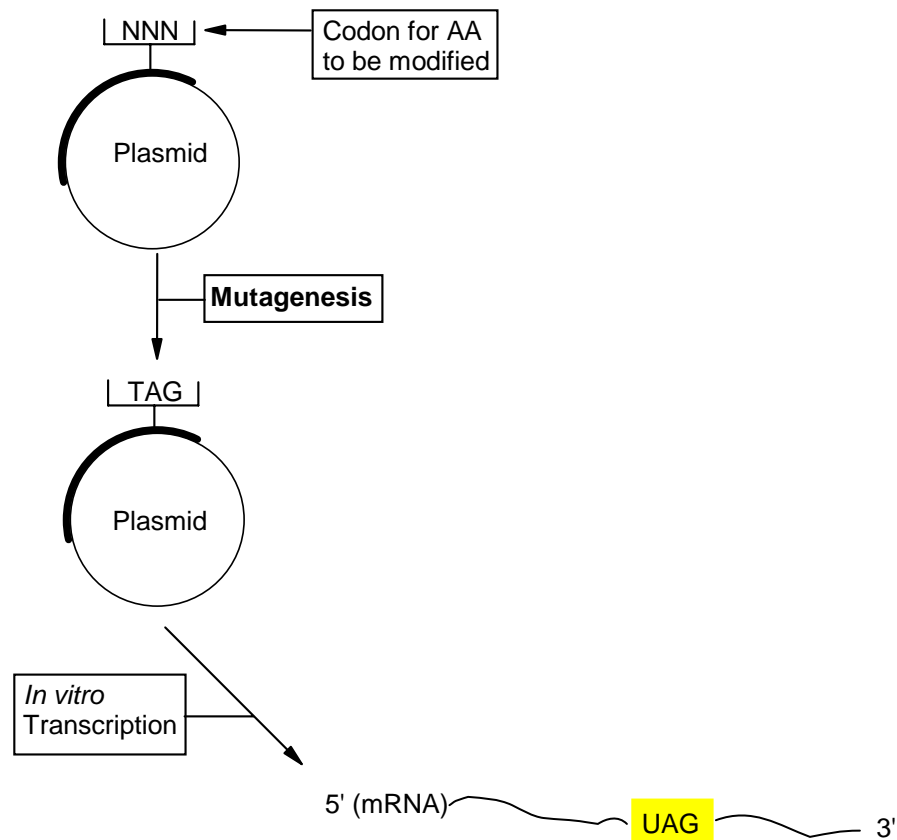
- Methods:
- (1) Attachment of Modified Amino Acid to tRNA
 - (a) Codon/Anticodon recognition is independent of AA attached to tRNA
 - (b) Translation System exhibits broad substrate specificity
 - (2) + Extension of the Genetic Code
 - (c) Genetic Code has 3 stop codons (two are available for extensions)

Genetic Code and Possible Extensions

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Term	UGA	Term
UUG	Leu	UCG	Ser	UAG	Term	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

L. Bossi, al.
S. M. Hecht, al.
P. G. Schultz, al.

Construction of Modified mRNA



REGIOSPECIFIC INCORPORATION OF GLYCOSYLATED AMINOACIDS INTO PROTEINS

Methods: (1) Attachment of Modified Amino Acid to tRNA (a) Codon/Anticodon recognition is independent of AA attached to tRNA
 (b) Translation System exhibits broad substrate specificity

(2) + Extension of the Genetic Code

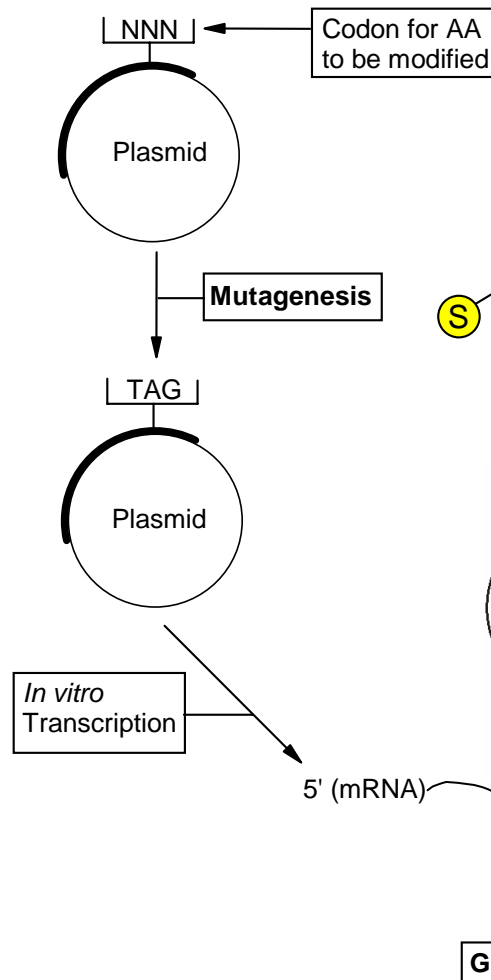
(c) Genetic Code has 3 stop codons (two are available for extensions

(d) Suppressor tRNAs with stop codon recognition exhibit efficiency *in vivo* and *in vitro*

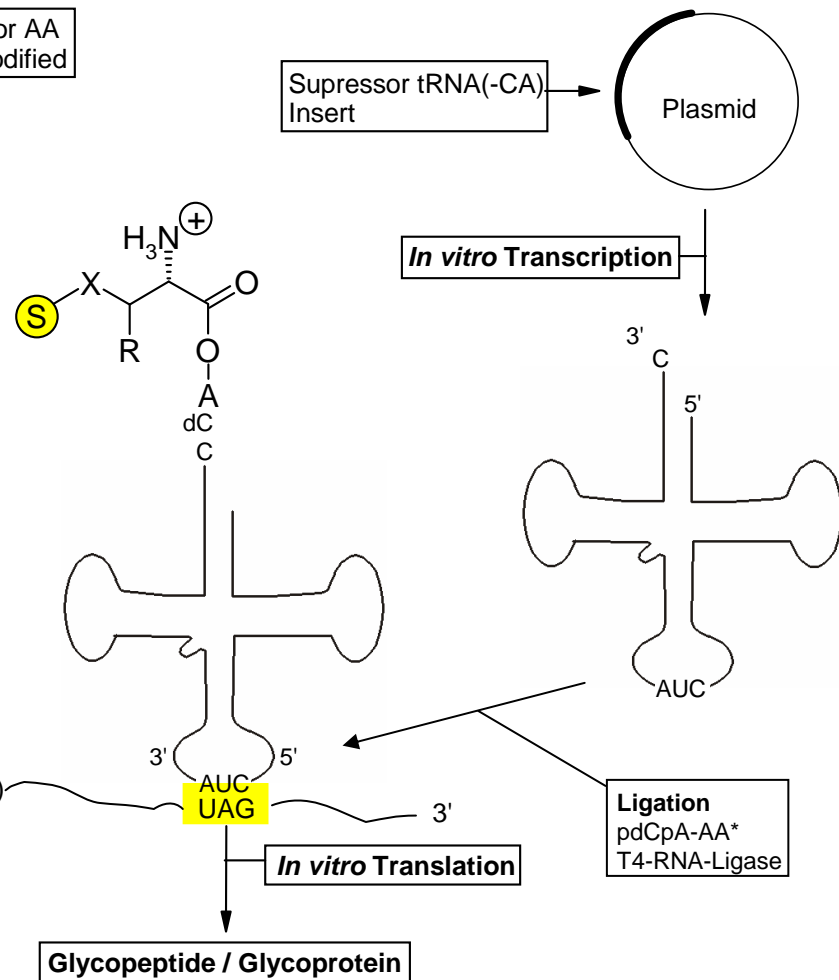
Genetic Code and Possible Extensions

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Term	UGA	Term
UUG	Leu	UCG	Ser	UAG	Term	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Construction of Modified mRNA

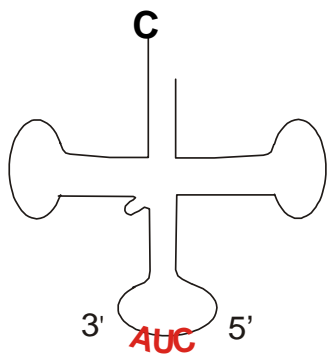
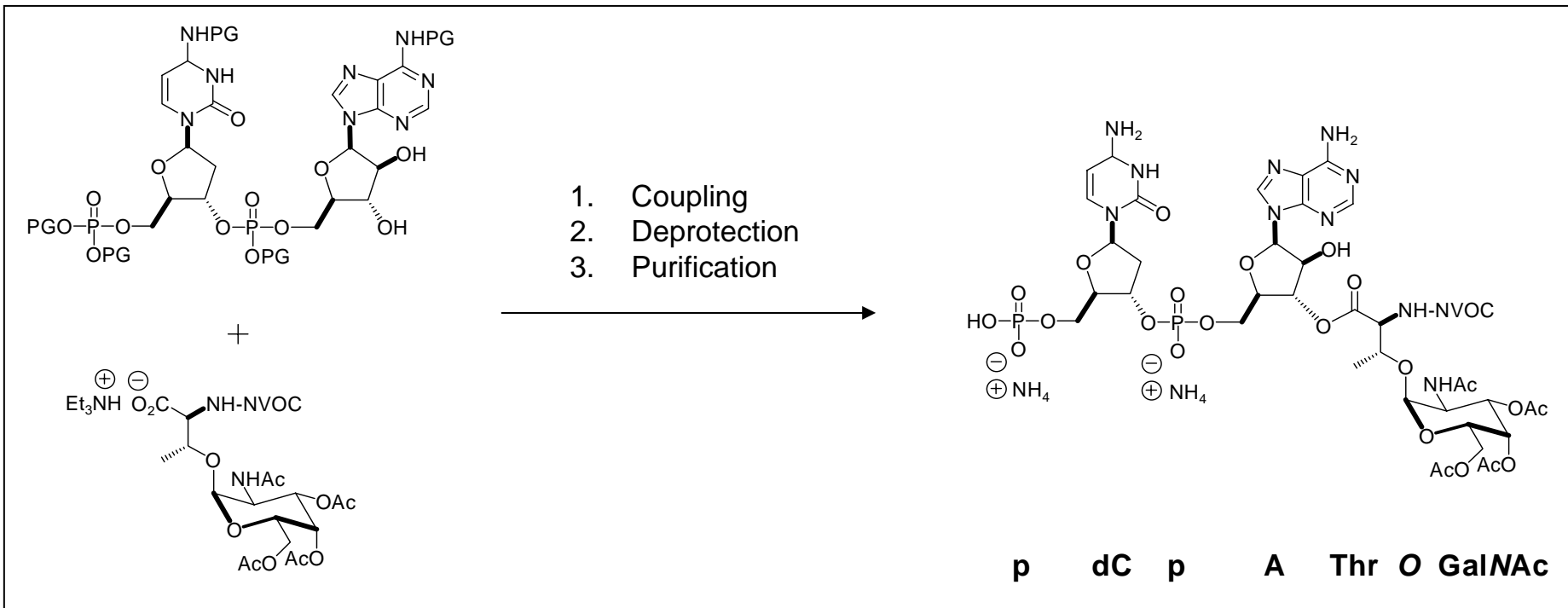


Construction of Suppressor-tRNA

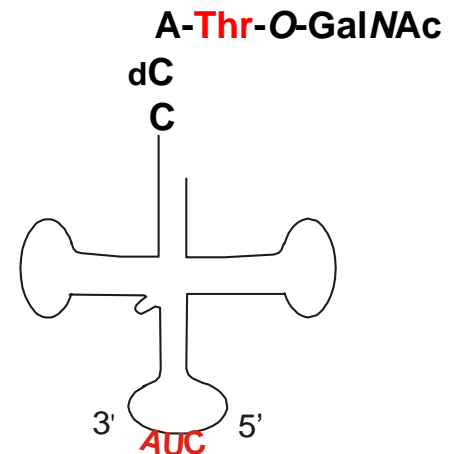


L. Bossi, al.
 S. M. Hecht, al.
 P. G. Schultz, al.

SYNTHESIS OF SUPPRESSOR tRNA^{Thr-GalNAc}

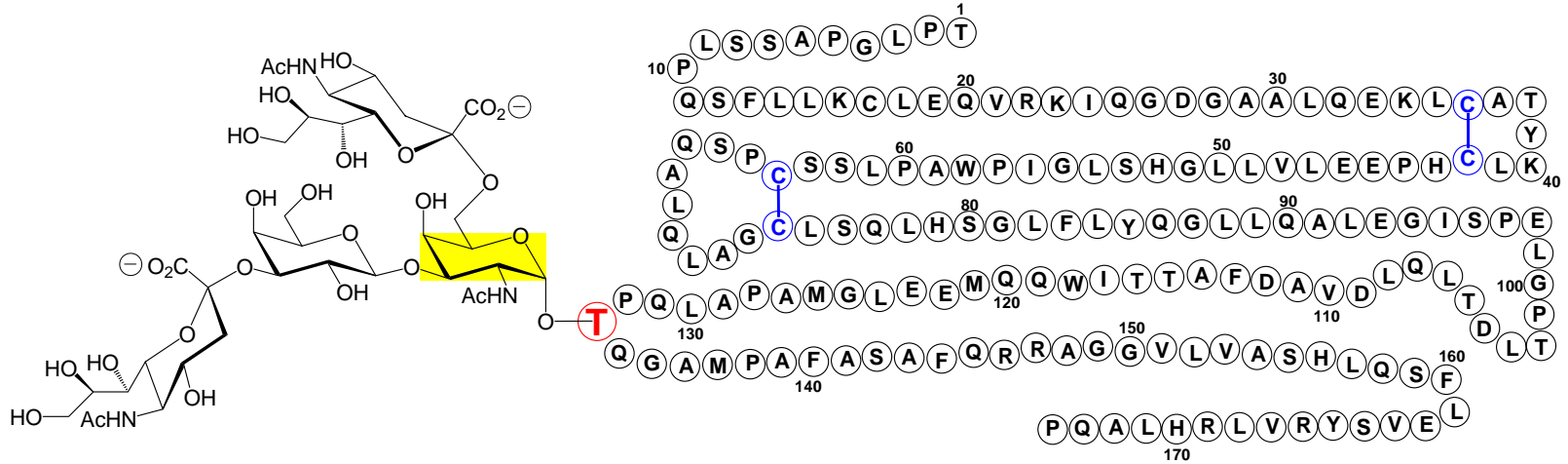


1. Ligation:
T4 RNA Ligase, ATP
pdCpA-Thr-O-GalNAc
2. NVOC cleavage:
hv, H₂O



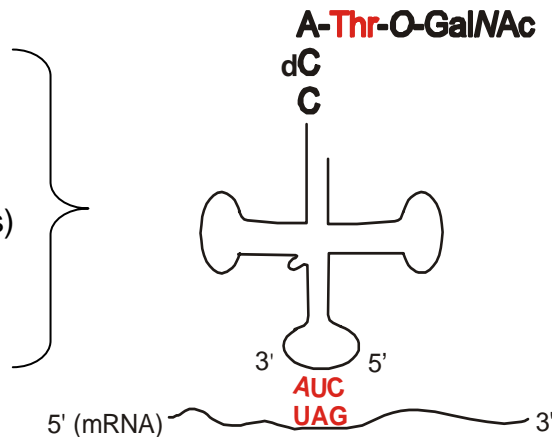
INCORPORATION OF N-ACETYL GALACTOSAMINE IN hG-CSF VIA NONNATURAL AMINO ACID MUTAGENESIS

hG-CSF (human granulocyte-colony stimulating factor) is an O-glycosylated interleukine

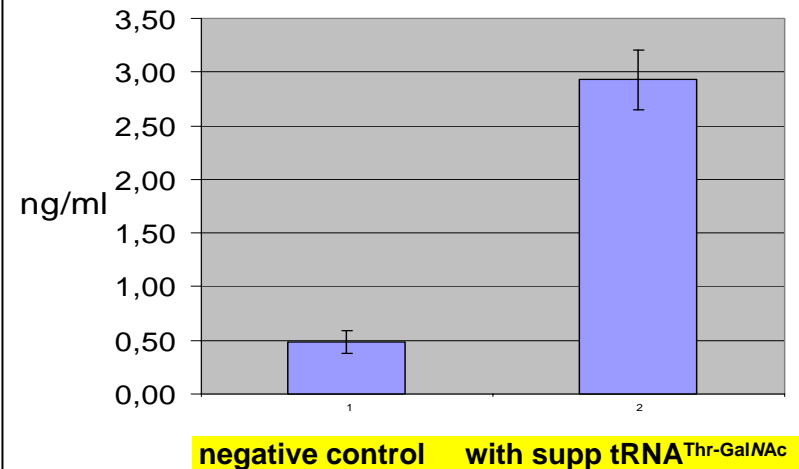


in vitro translation with suppressor tRNA^{Thr-GalNAc}

- 1) amino acids
- 2) buffer
- 3) rabbit reticulocyte lysate (ribosomes, tRNAs, ARSs)
- 4) mutated G-CSF mRNA
- 5) suppressor tRNA^{Thr-GalNAc}



Product analysis with hG-CSF ELISA





16.12.1999

The Age of Biology?

Here at the threshold of a new millennium, the pace of **scientific and technological advance seems overwhelming**. Computers are obsolescent the moment we buy them. New discoveries at the farthest reaches of the universe leave us awestruck. Scientists are racing to delineate the entire human genome ...

Our predecessors felt just as breathless at the end of the last century ... But that was just a warm-up. The hallmark of this century – from splitting the atom to cracking the genetic code – has been a faster advance ... than ever before in history ...

In **a famously wrong pronouncement back in 1899**, the head of the U.S. Patent Office suggested that **everything important had already been discovered**. Now we are hearing some of the same talk, ...

Indeed, if the **past century** has been the **age of chemistry and physics, the next will probably be the age of biology**.

Cooperation of Biology, Chemistry, Physics!!!