# Rational Design of Vaccine Adjuvants Based on Dendritic Cell Receptor Agonists;

## Harnessing Innate Immunity for The Development of New Generation Vaccines

**VTH-2015, 2nd October, 2015**

"Immunization has been a great public health success story. The lives of millions of children have been saved, millions have the chance of a longer healthier life, a greater chance to learn, to play, to read and write, to move around freely without suffering".

*Nelson Mandela,*



**HALMUTHUR SAMPATH KUMAR**
**Vaccine Immunology Laboratory,**
**CSIR-IICT, Hyderabad , India**

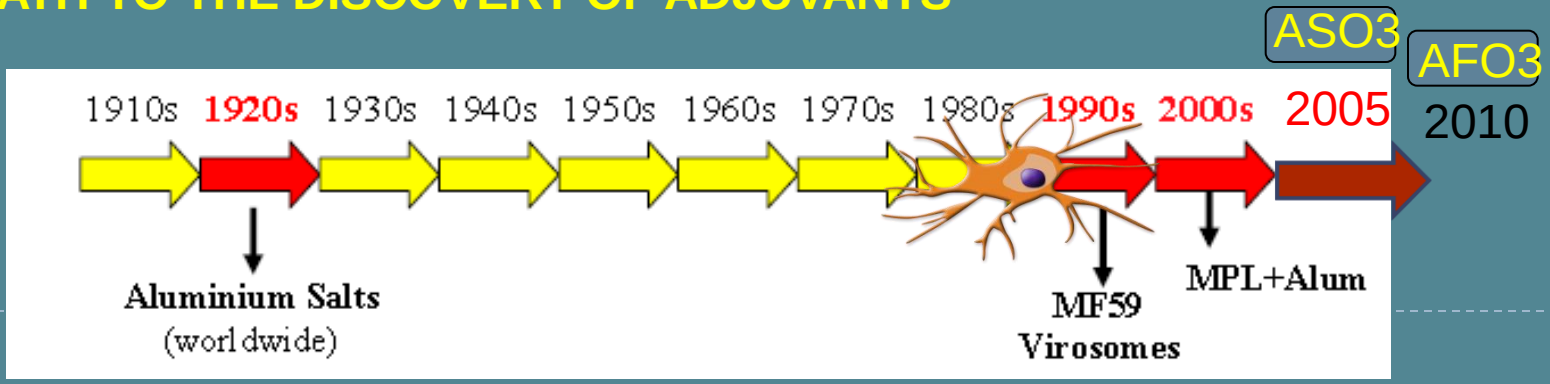
# Why do we need Adjuvants??

**Definition: An adjuvant is a chemical substance that can be added to a vaccine in order to enhance immune response.**

- Traditional self adjuvanted vaccines based on attenuated live organisms being invasive in nature provides efficient delivery to antigen-presenting cells and Various naturally occuring components of the pathogens stimulate the innate immune system

- The majority of recent vaccines represent highly purified subunit components of pathogens lack most of the features of the original pathogens, such as immunostimulatory components, and the ability to replicate and produce high level of antigens, Therefore, they are usually poorly immunogenic and need adjuvants to improve immunogenicity.

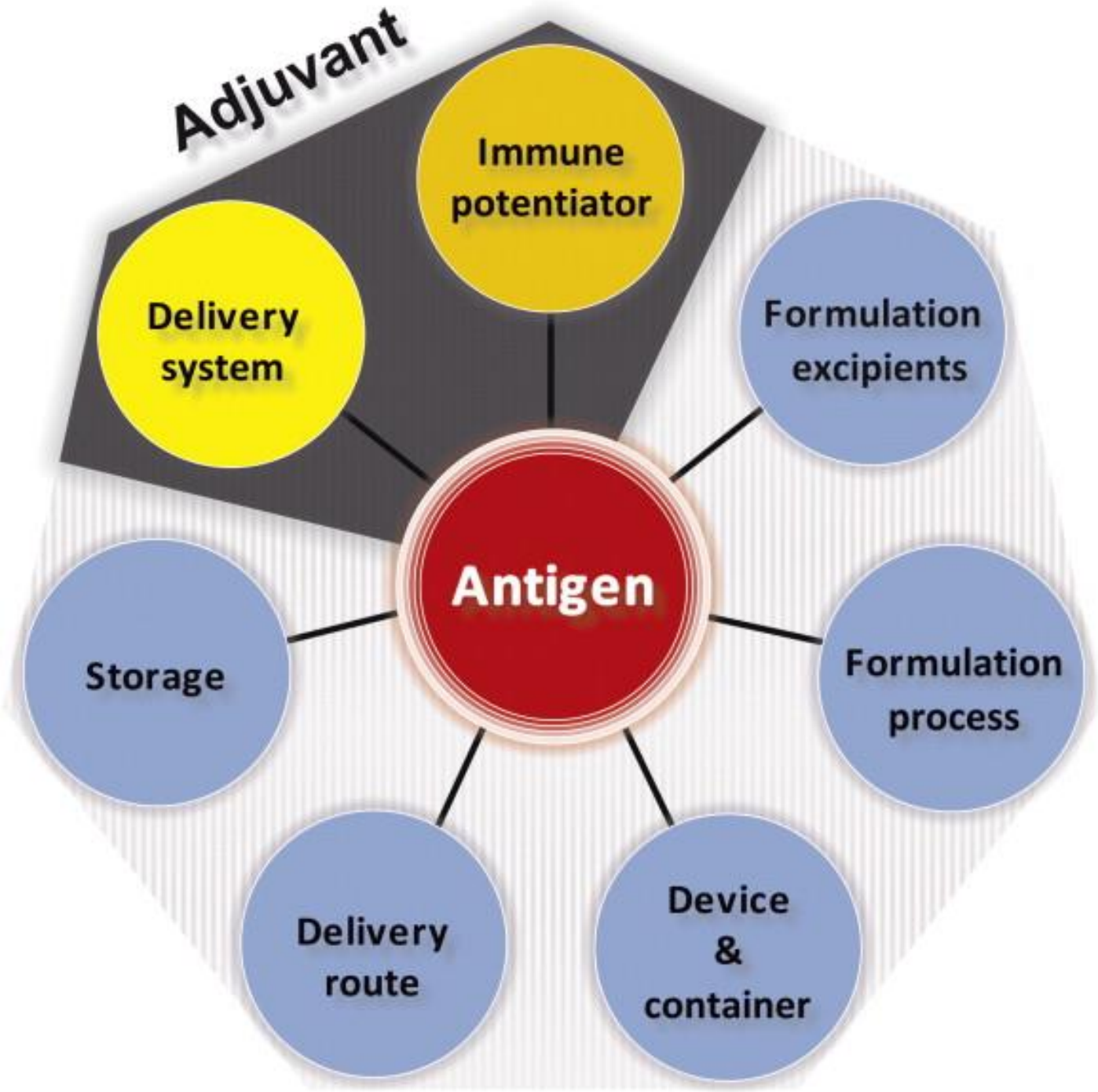
**SLOW PATH TO THE DISCOVERY OF ADJUVANTS**



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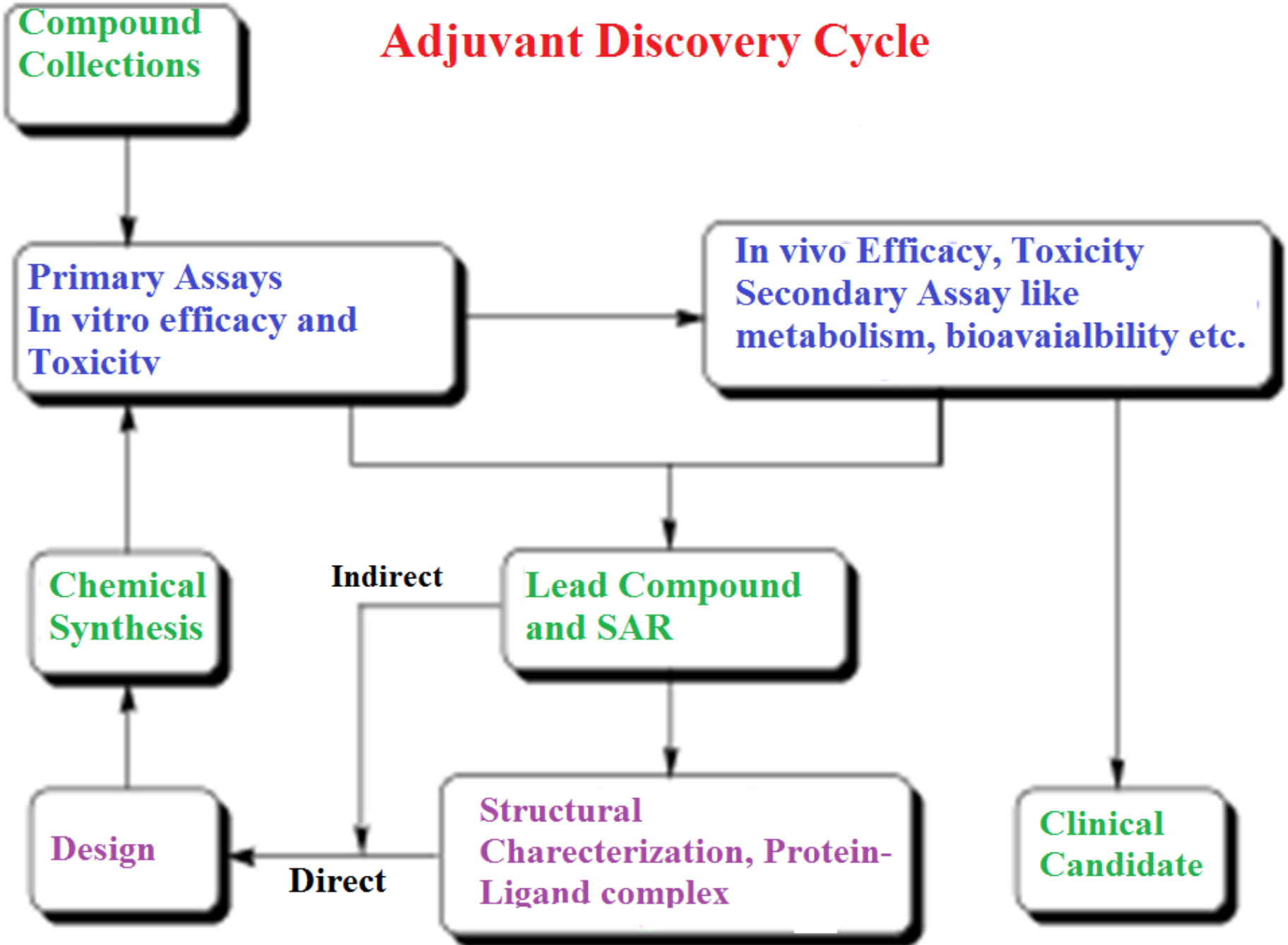



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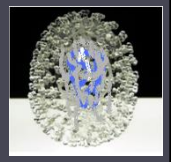
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# Small Molecules as Vaccine Adjuvants Natural , Semisynthetic, Synthetic

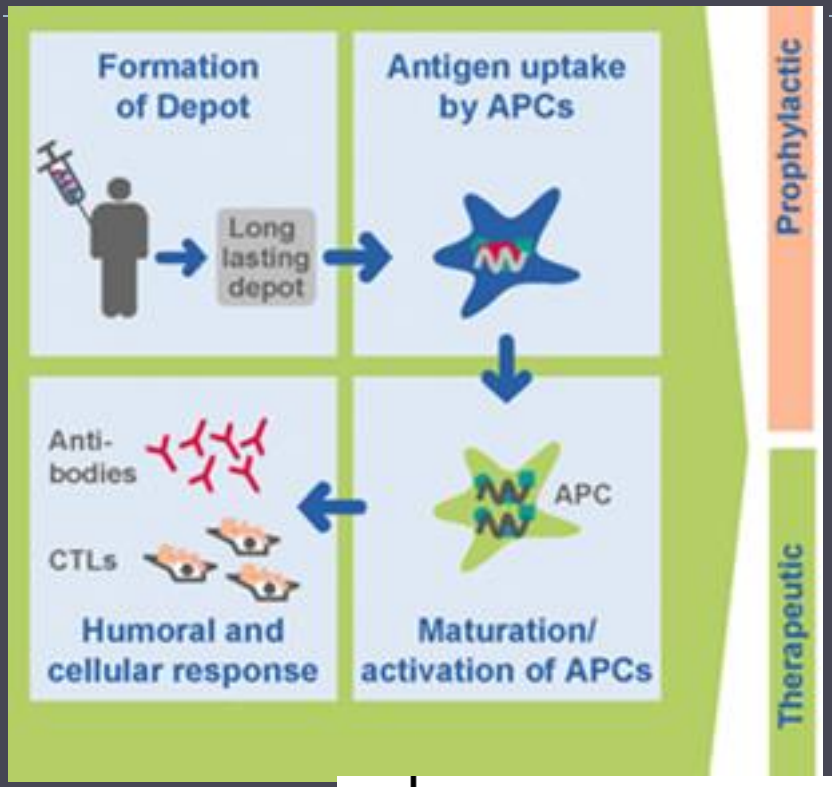


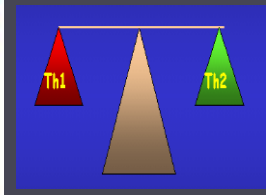
# Adjuvant Design-Multiple Roles of Adjuvants

## Multiple Roles of Adjuvants



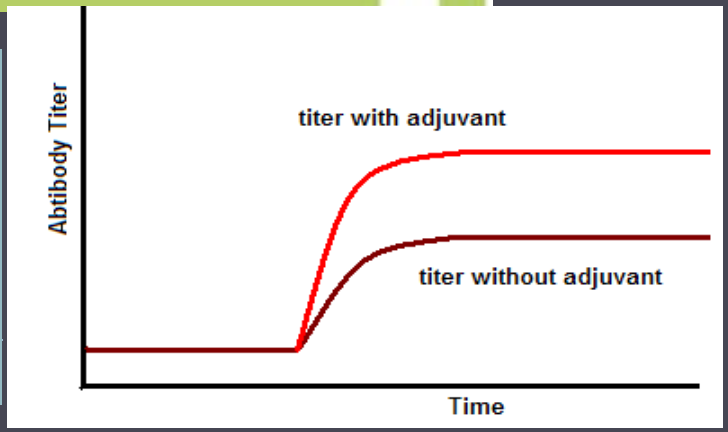
- Depot Formation
- Rapid antigen delivery into APC
- Effective antigen processing/presentation
- Humoral and cell mediated immunity
- Immune Memory
- Complimentary immune response to specific antigen




## Benefits of Adjuvants

- **Replacement of Live Vaccine with Subunit Vaccine- New Vaccines**
- **Permits use of a much smaller quantity of Antigen.- Affordable**

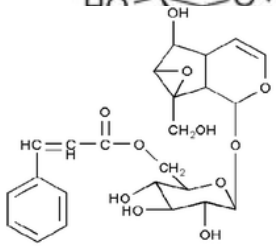


# Plants with Immunomodulatory Potential as possible source of Adjuvants ??

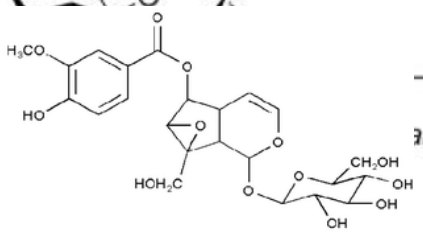



QS-21 (Quillaja saponaria)



Picroside I

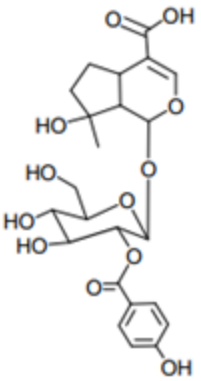


Picroside II

GSK ASO2



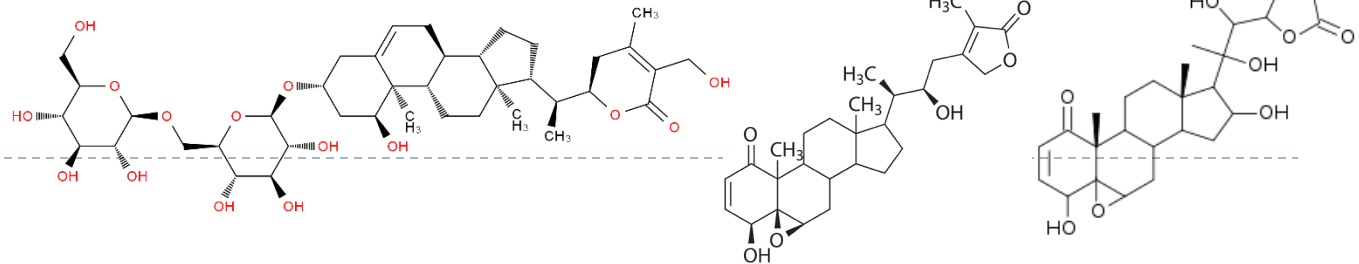
Picrorhiza kurroa



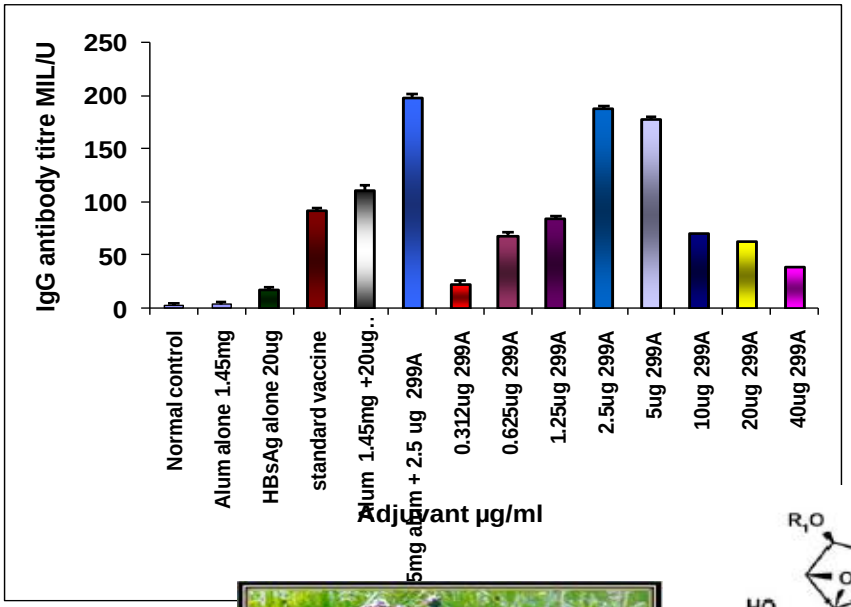


Vitex negundo Linn



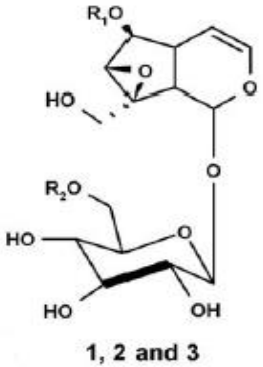



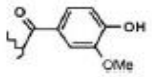
Withania somnifera

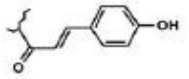




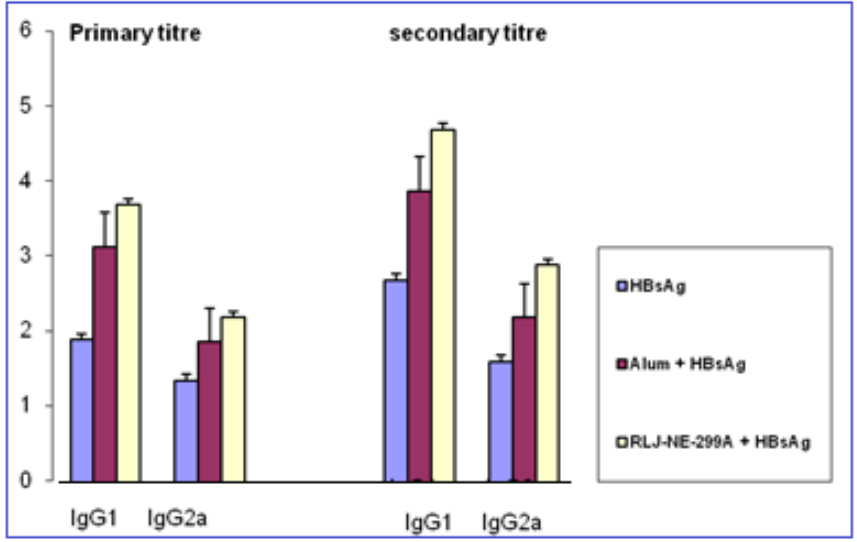


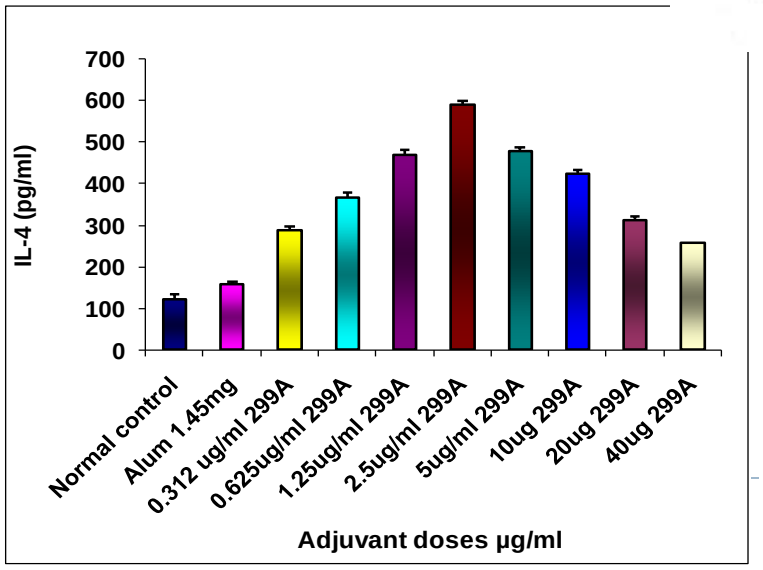


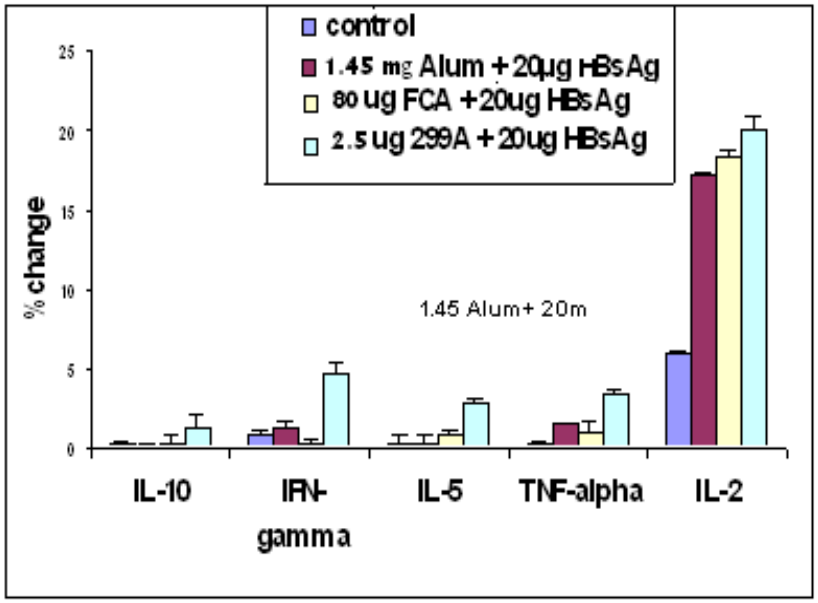

1. Picroside II (PK-II) = $R_1$ -  $R_2$ - H

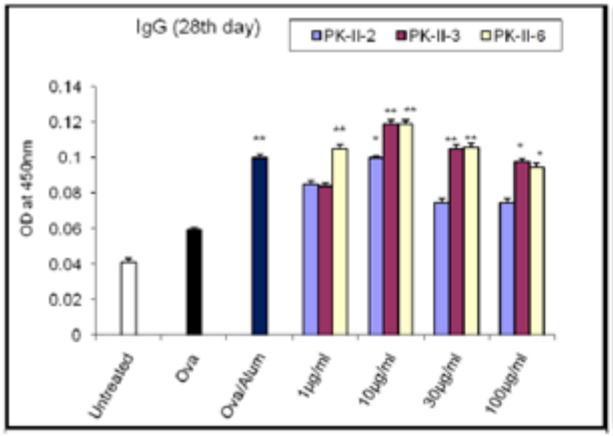
2. Picroside I (PK-I) = $R_1$ - H $R_2$ - 

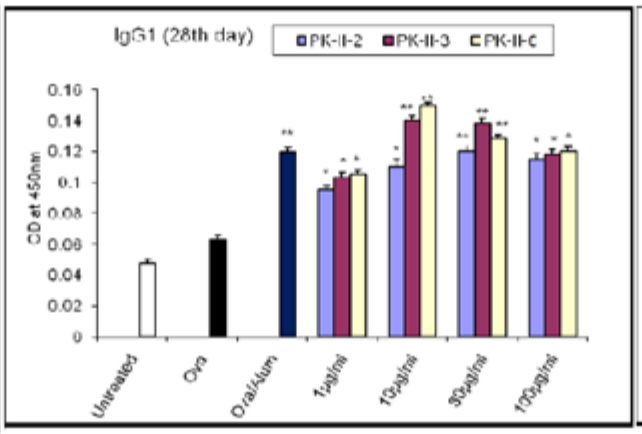
3. Catalpol = $R_1$ - H $R_2$ - H

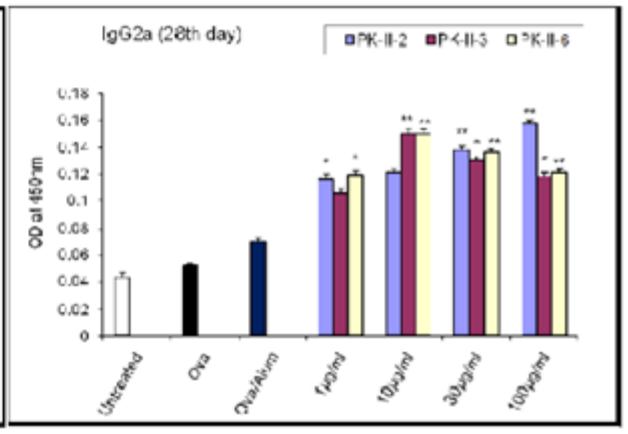


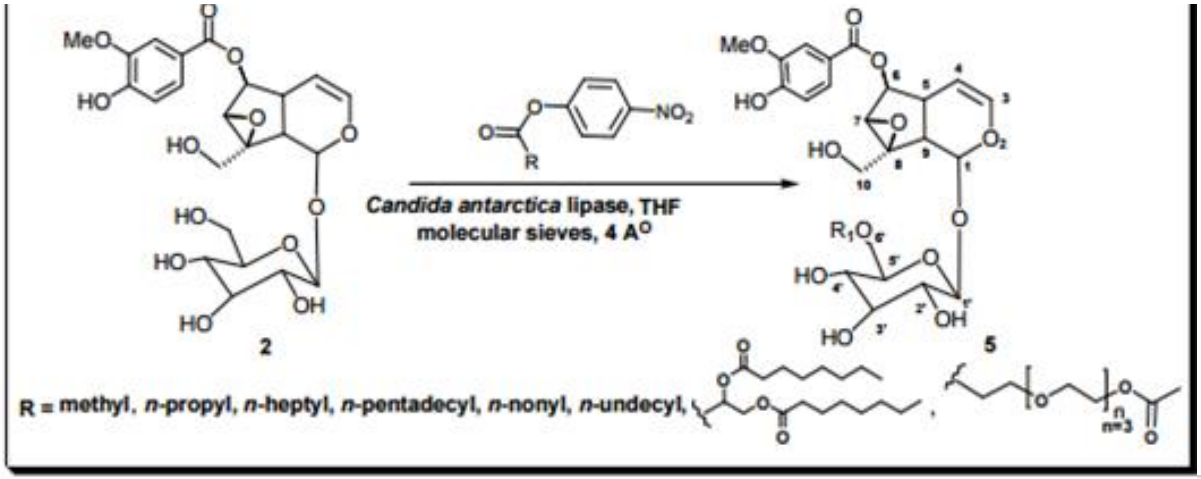



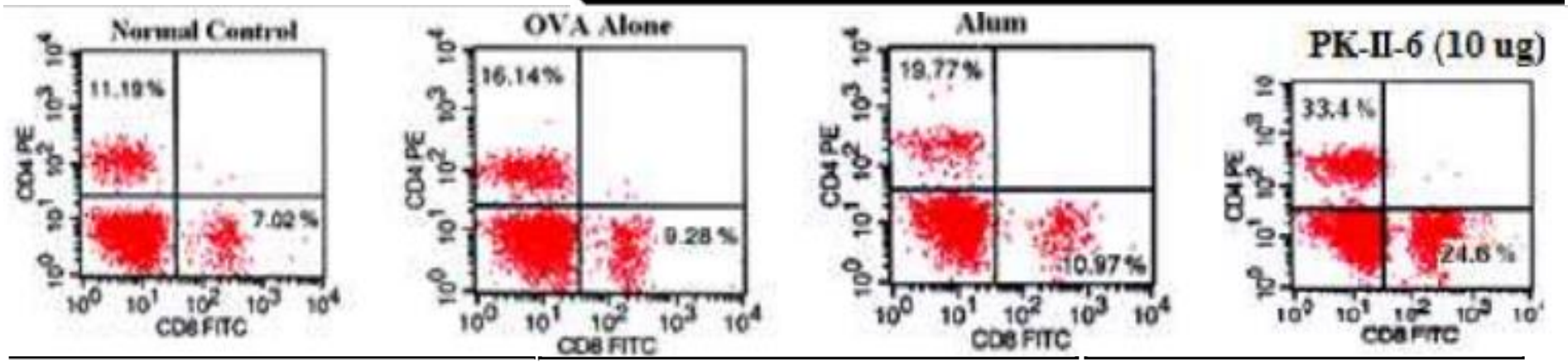


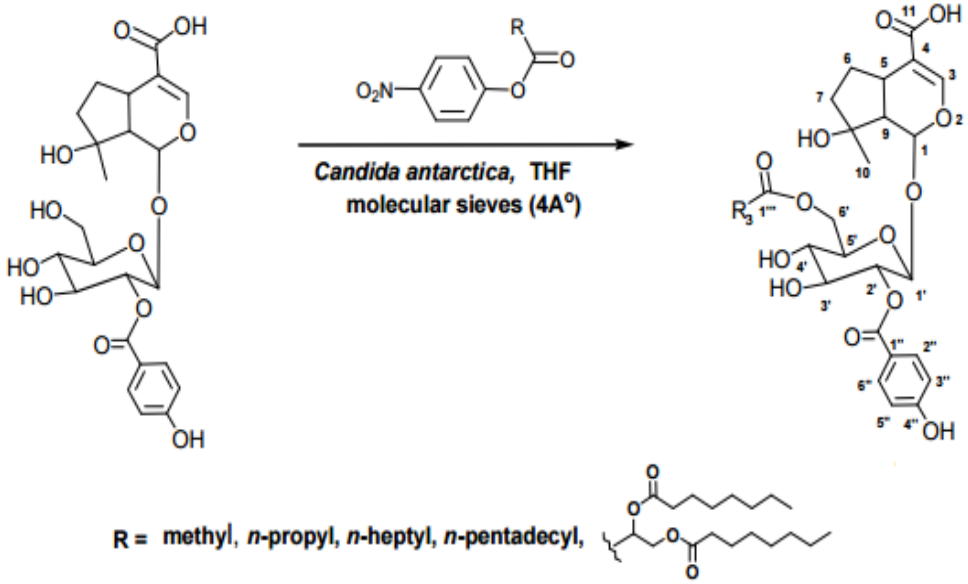

## Lipase Catalysed Regioselective Acylation of Picroside-II



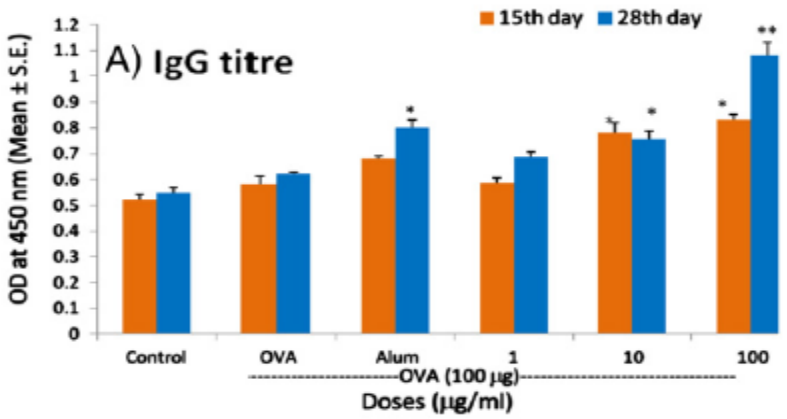

Halmuthur Kumar et al., *Vaccine*, 28 (2010) 8327–8383

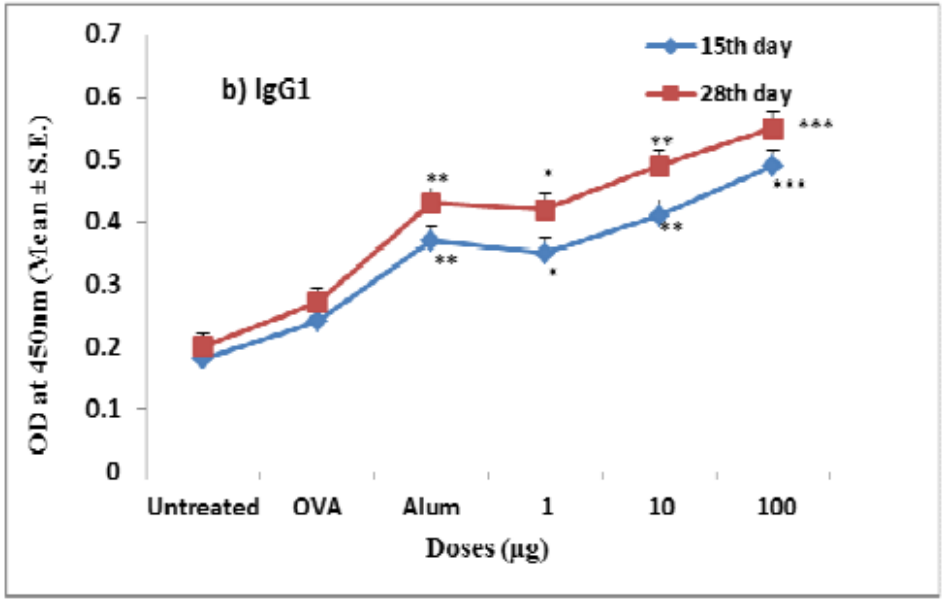
## Regio-selective acylation of Agnuside

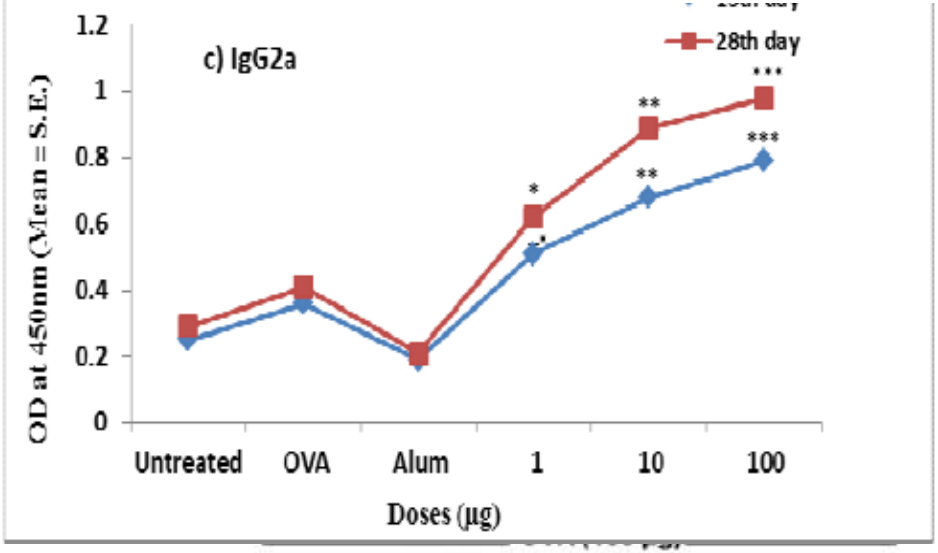









Doses (µg/ml)

**Parvinder Singh and Halmuthur Kumar et al.,**
***International Immunopharmacology* 11 (2011) 1855–1863**

# Plant Based Adjuvants:
## Drawbacks

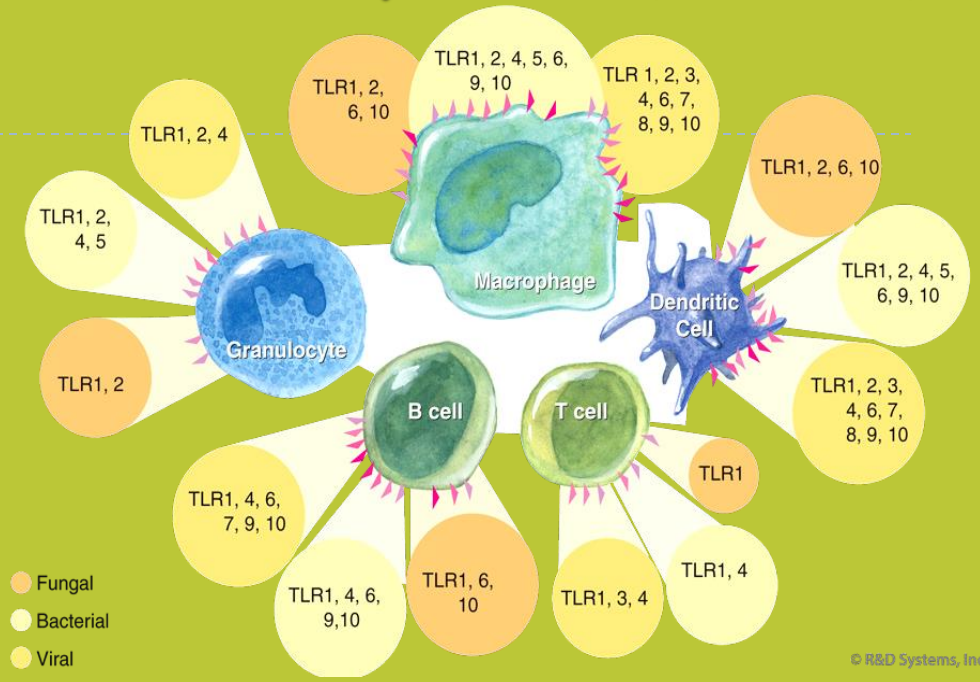
- Availability of plant in abundance
- Isolation process to be economical

- Alternative to expensive adjvants from plant sources
- In expensive Synthetic route to plant based adjuvants QS21 is highly expensive
- Scaffold modification a promising strategy
- Mode of action to be established



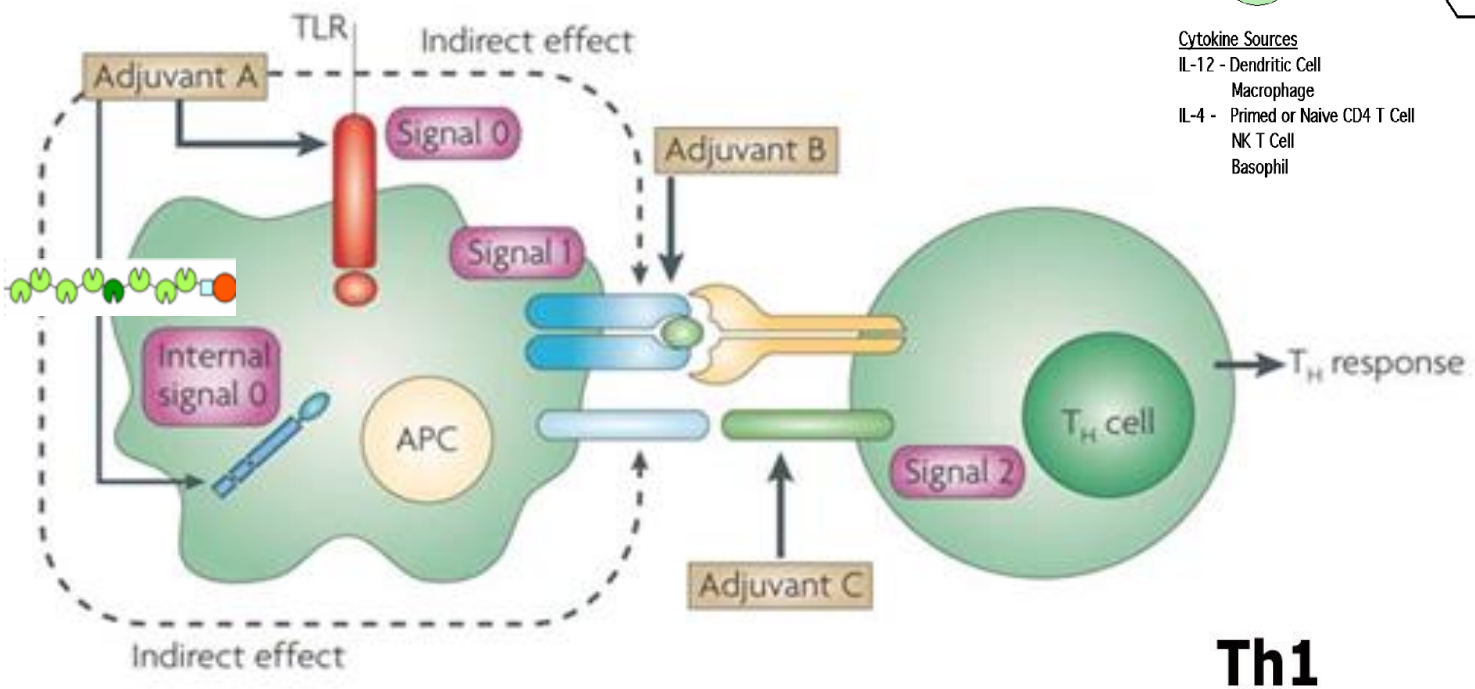
# Pattern Recognition Receptors (PRRs)

- Toll-like receptors
- NOD /RIG receptors
- Mannose binding lectin (MBL)
- C-reactive protein

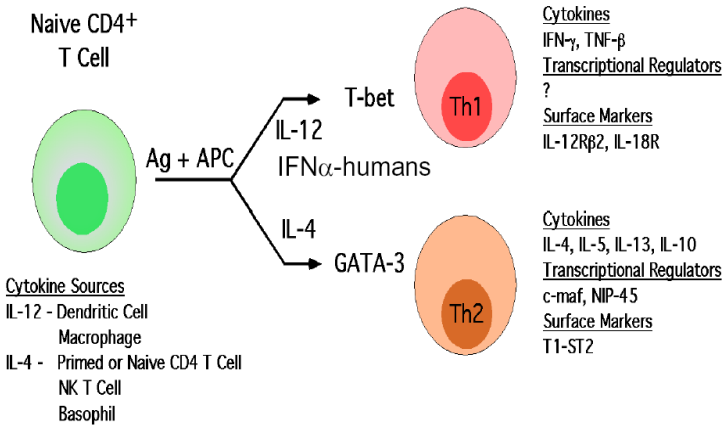



- Functions of PRRs:
  - Opsonization, activation of complement and coagulation cascades,
  - Phagocytosis,
  - Activation of pro-inflammatory signaling pathways,
  - Apoptosis

# Strategy for Harnessing Innate Immunity




## Cytokines are the Principal Regulators of $T_H1/T_H2$ Polarization





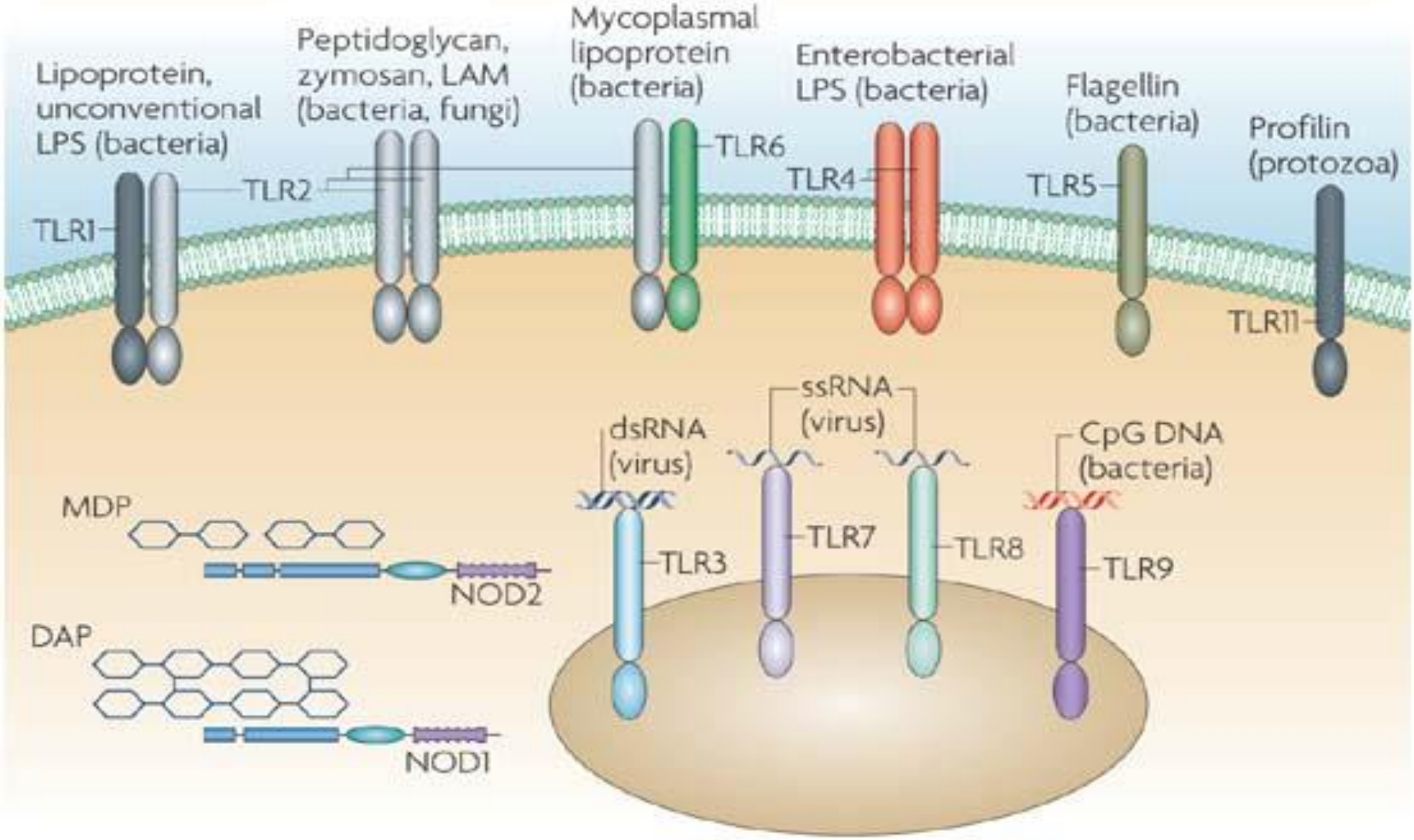


- Combination of delivery system and TH1 effectors
- Safe adjuvants with reduced toxicity- Modify known l

# PAMPS

- **Conserved** among microbes
- Known as **P**athogen-**A**ssociated **M**olecular **P**atterns (**PAMPs**)
- PAMPs are recognized by **plants** as well as **animals**, meaning this innate response arose before the split
- Recognized by the complimentary **PRR**
- **Only** vertebrates have evolved an adaptive immune response

# Dendritic cell TLRs and their Agonists



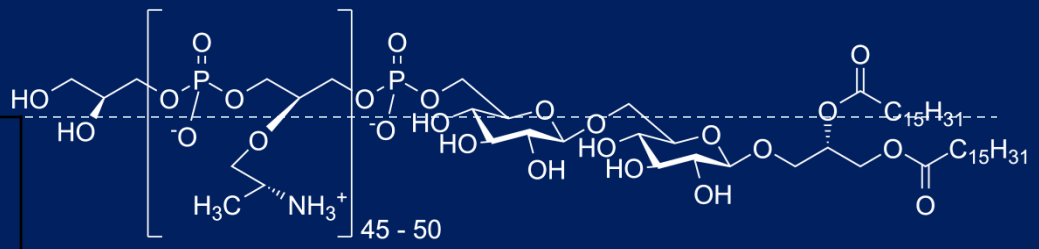
# Microbial Pattern Recognition Ligands

**Virtues**

- Powerful PRR specificity
- Humoral and cell mediated mmunity
- High activity at low doses
- Overall Th1/Th2 balance

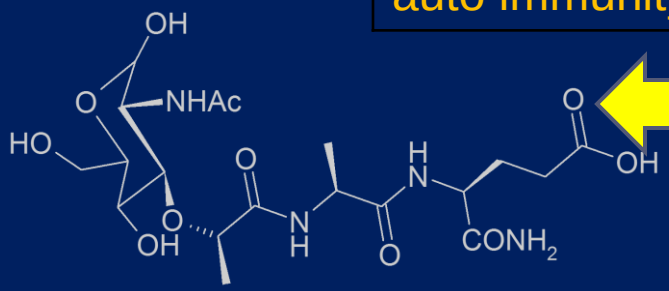
**Drawbacks**

- High pyrogenicity
- High Toxicity
- Often induce auto immunity

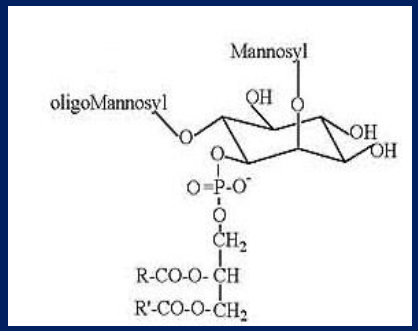



Lipoteichoic acid(LTA)

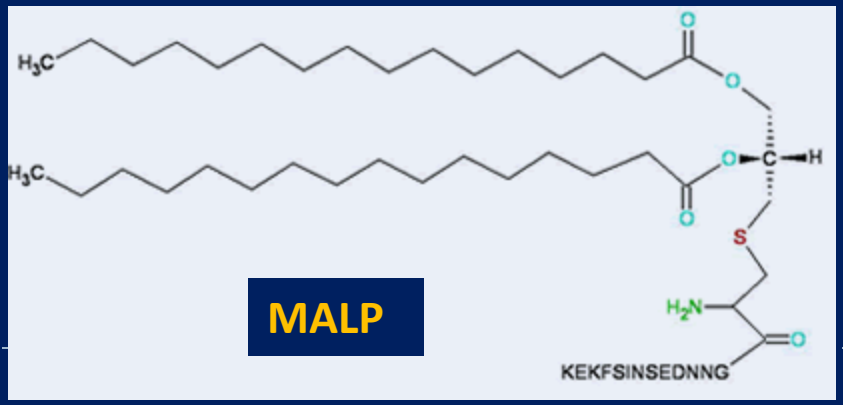
PAMP



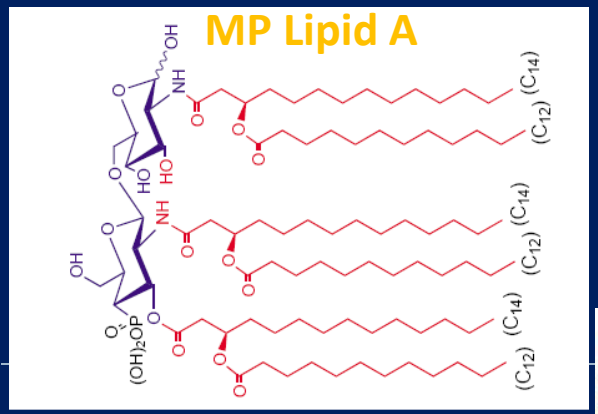
MDP



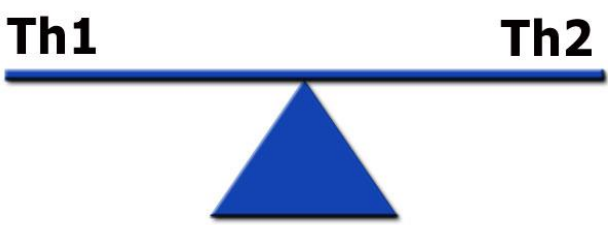

PIMS



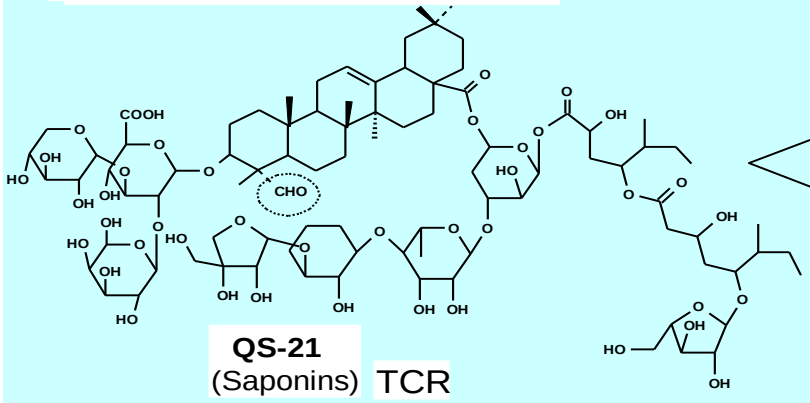

MALP



MP Lipid A

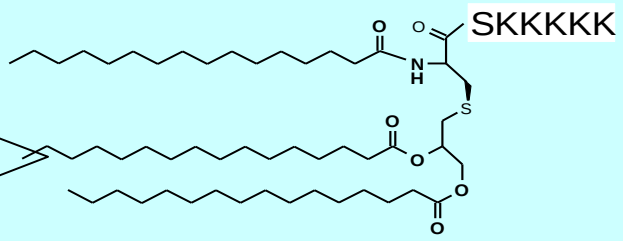



# Immune response derived from select PAMPs and TCR Ligands

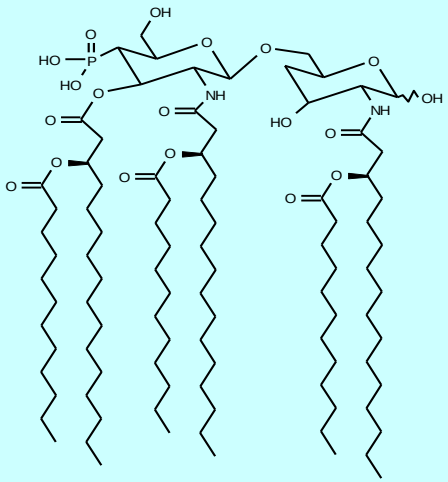



**QS-21**
(Saponins) TCR

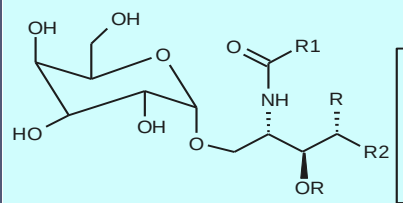
Th1
IL2, IFN-γ
Th2
IL-4,5,10
IgG, CD8+




**Pam3cys**
(Lipopeptide)
TLR2

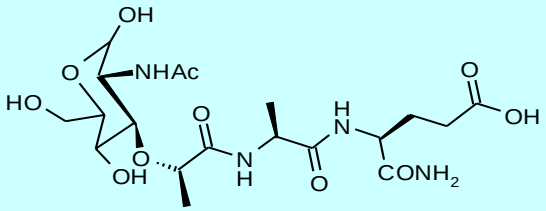


**Monophosphoryl lipid**
(Liposaccharide)
TLR-**4**, 2



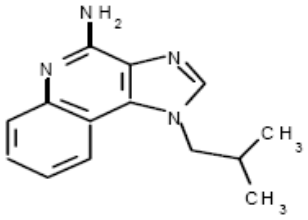

Th2
IL-4,5,10
IgG, CD8+

**α-Galactoceramide**
(spingolipid)
NK Cell α,β-TCR



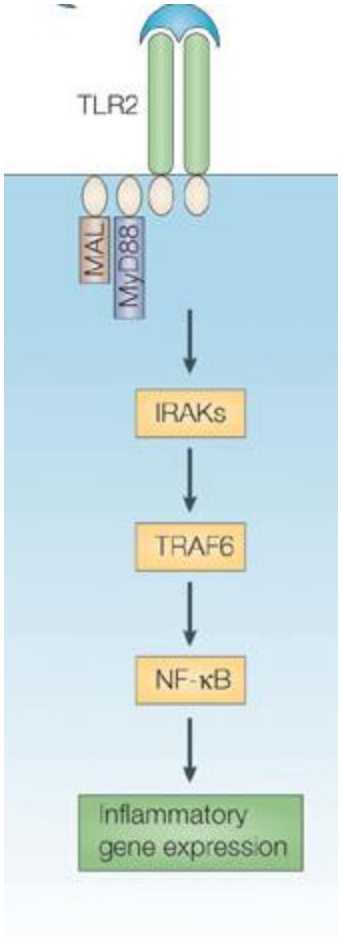

**Muramyl dipeptide**
(Glycopeptide)
TLR 4, 2
NOD-2

Th1
IL2, IFN-G
TNF-α
Th2
IL-4, 6
IgG, CD8+

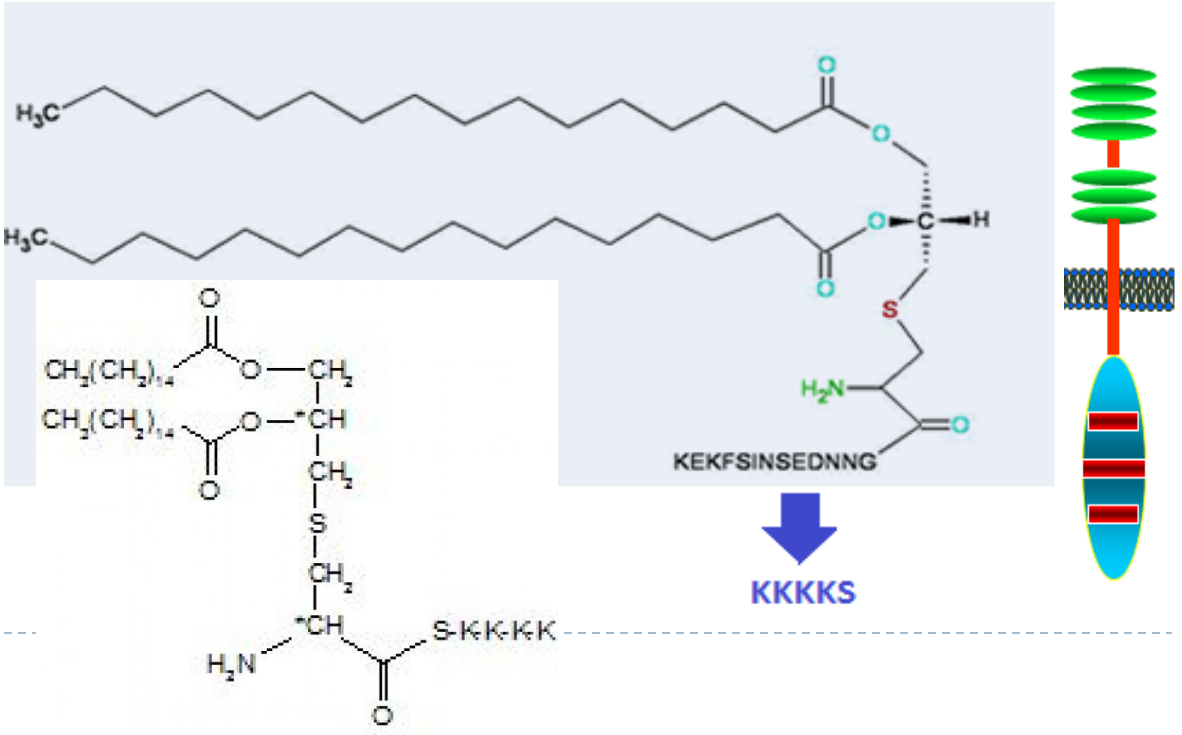



**Imiquimod**
(Imidazoquinoline)
TLR7/8

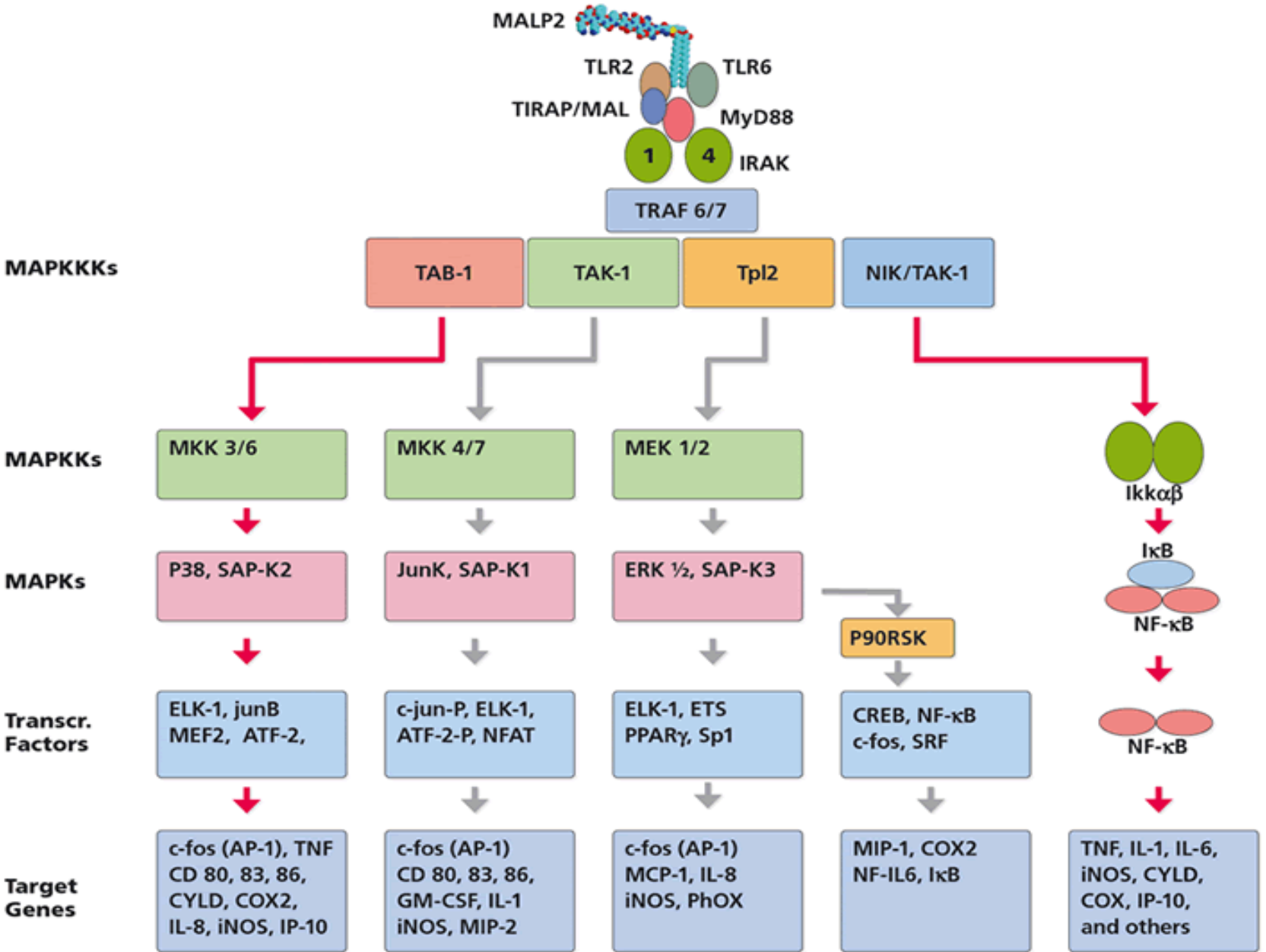
# LIPOPEPETIDE BASED-TLR2 AGONIST



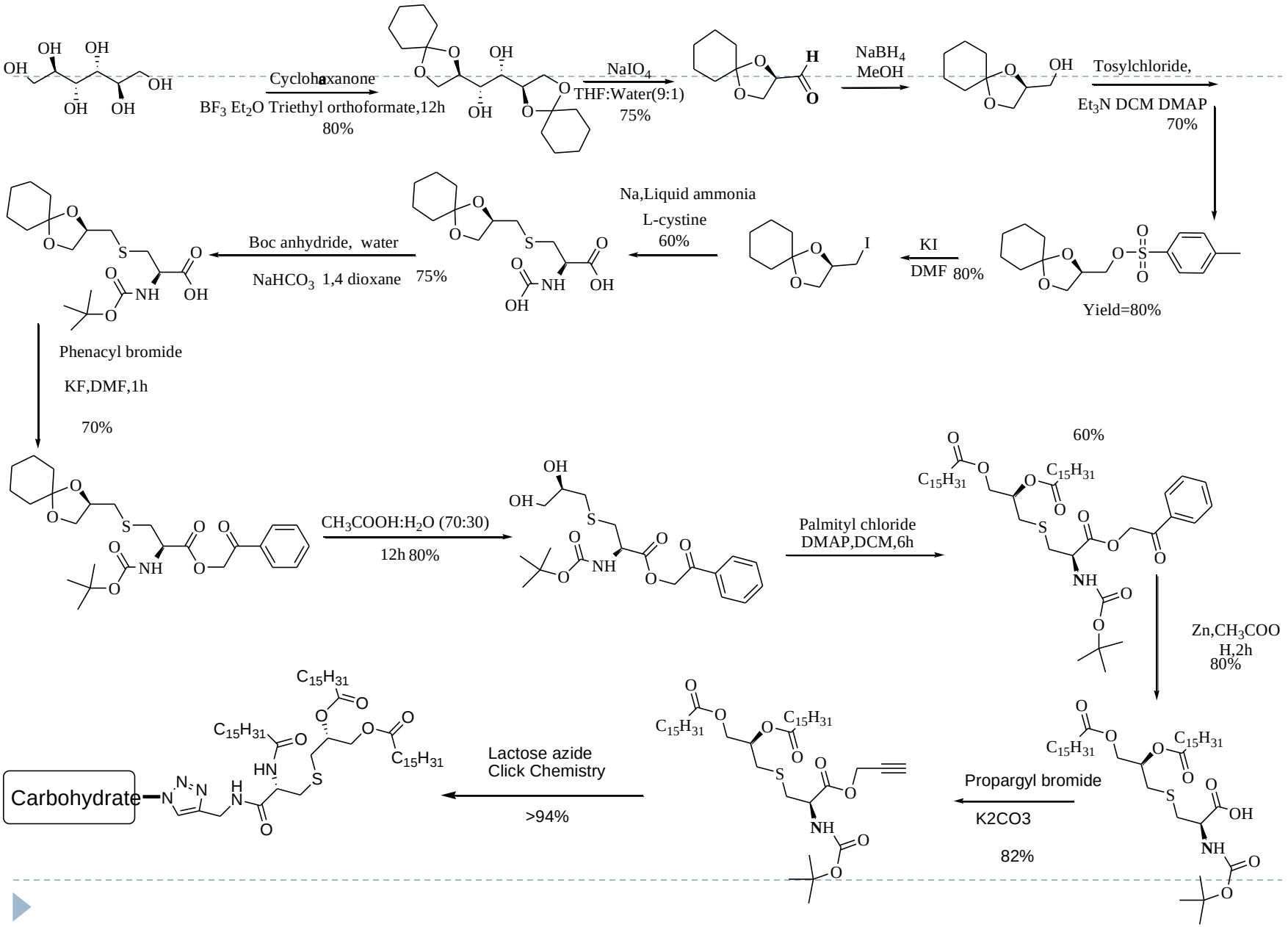

- Synthetic lipopeptides like MALP have demonstrated great potential as a vaccine strategy for eliciting cellular and humoral immunity.
- **PAM3CYS** binds and activates dendritic cells by engagement of Toll like receptor 2 (TLR 2). as well as its less hydrophobic nature, indicate that PAM3CS could also be used as a self-adjuvanting moiety (Derived from *Micoplasma Fermentan*)



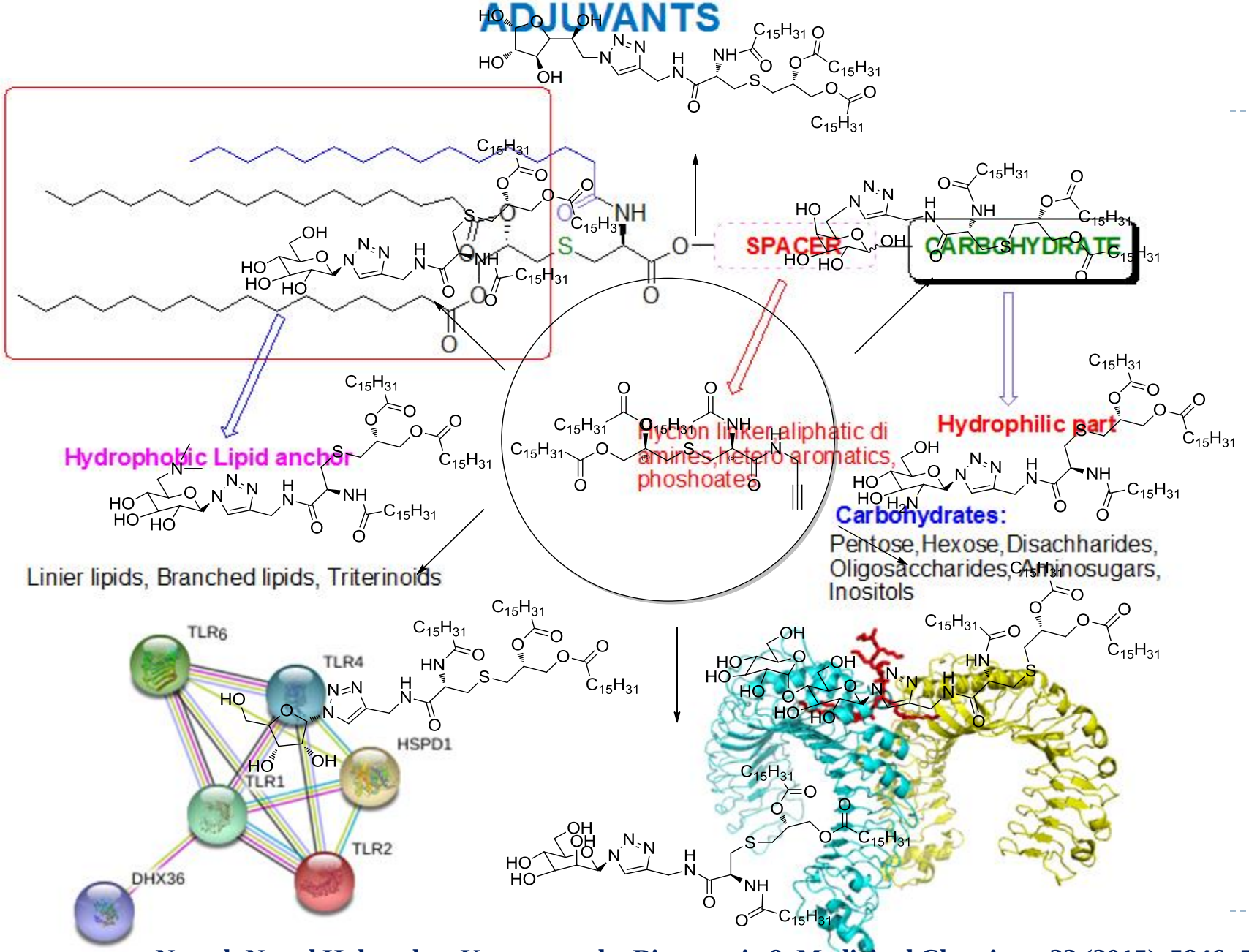
# Ligand field expansion through diversity oriented synthesis approach leads to adjuvants with varying degree of immunogenicity



# Click-Chemistry Approach to Novel triazole tethered carbohydrate Pam3Cys conjugates



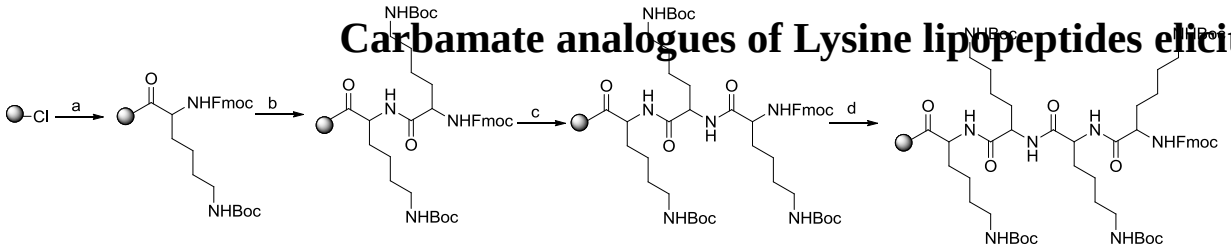
# Novel TLR2-Agonistic Triazole tethered Pam2Cys cabohydrate conjugates




Naresh N and Halmuthur Kumar. et al., Bioorganic & Medicinal Chemistry 23 (2015) ,5846–5855.

# Immunological Evaluation of Pam3Cys conjugates as TLR2 agonists

## Carbamate analogues of Lysine lipopeptides elicit Adjuvanticity



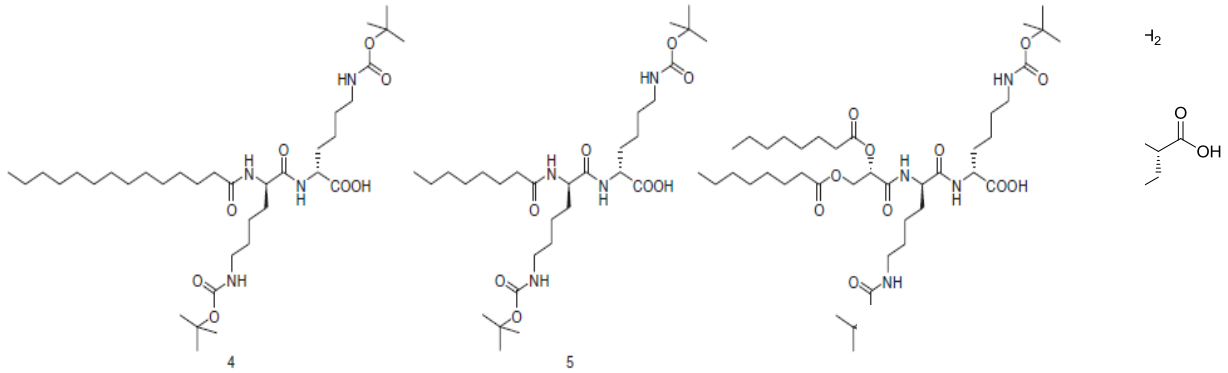
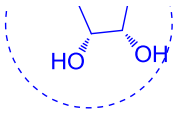
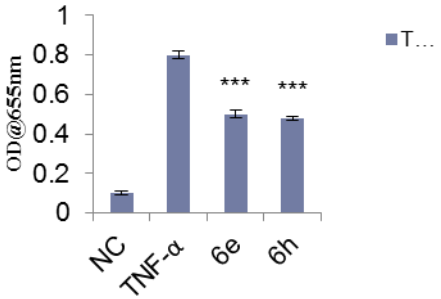



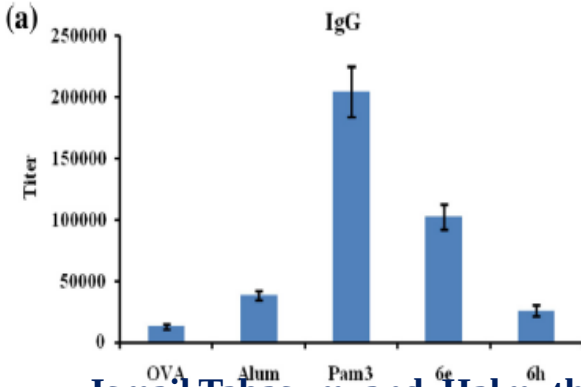

Fig. 1. Active dipeptide amphiphiles.

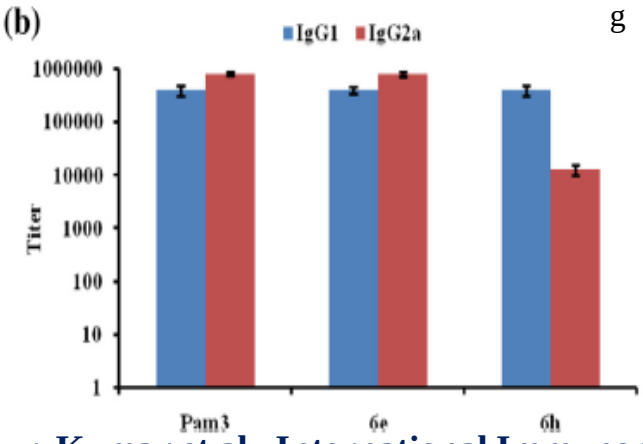



Human TLR-2 reporter gene assay

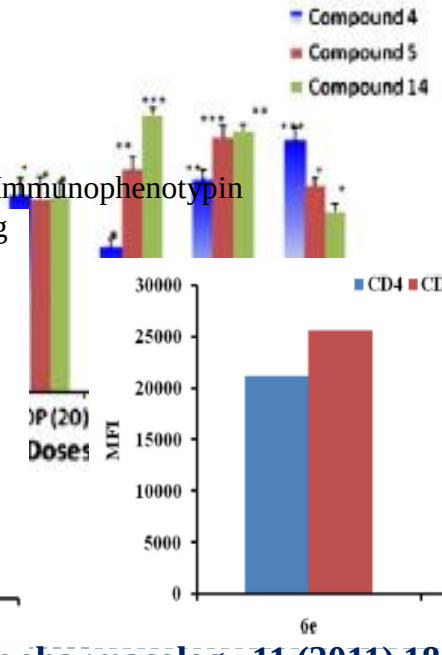



Antigen specific antibody response




Immunophenotyping

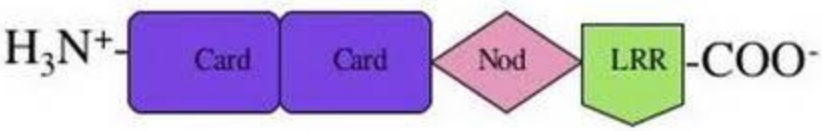


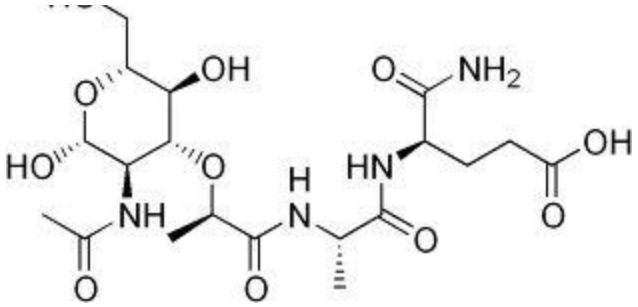


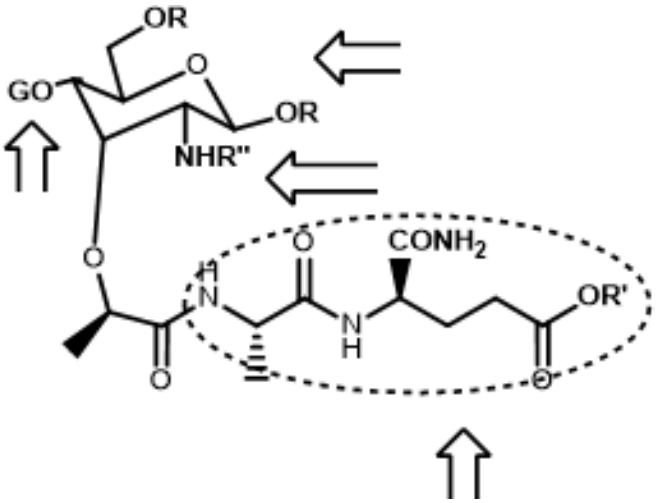
Ismail Tabasum and Halmuthur Kumar et al., International Immunopharmacology 11 (2011) 1855–1863

Naresh N and Halmuthur Kumar. et al., Bioorganic & Medicinal Chemistry 23 (2015) ,5846–5855.

# MDP and Derivatives-**NOD2 Agonists**



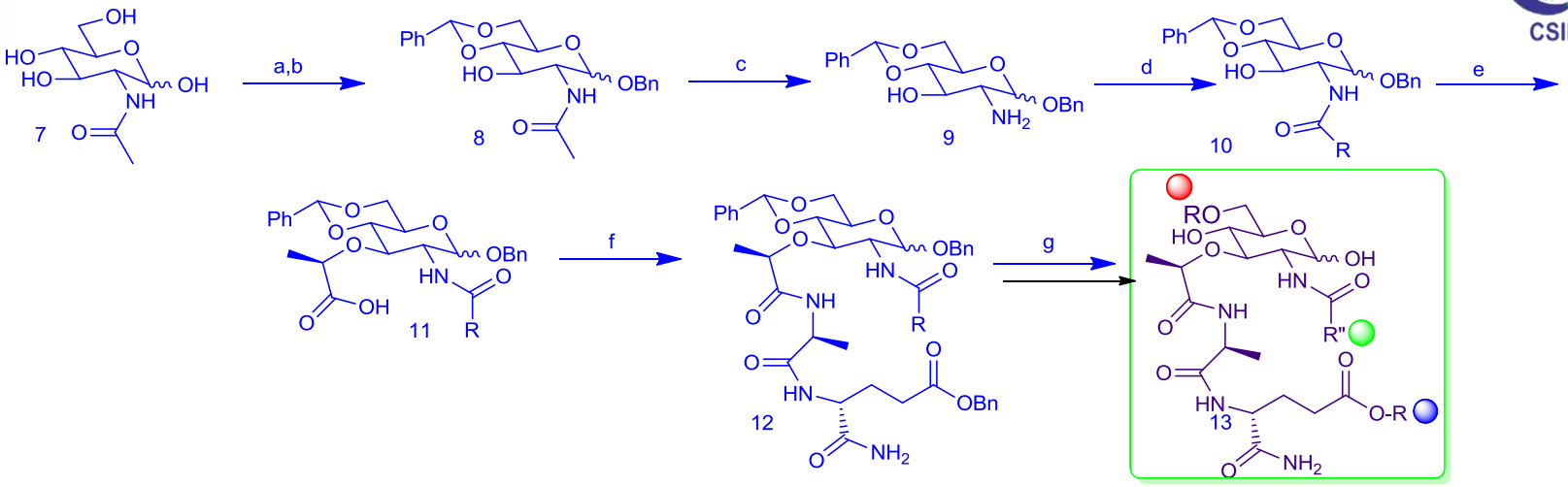




**MDP (MurNAc-L-Ala-D-isoGln, also known as muramyl dipeptide), is the minimal bioactive peptidoglycan motif common to all bacteria and the essential structure required for adjuvant activity in vaccines. MDP is recognized by NOD2 (CARD15) a member of the family of Nod-like receptors (NLRs, also known as CATERPILLER), characterized by a nucleotide-oligomerization domain (NOD) and ligand-recognizing leucine-rich repeats.**

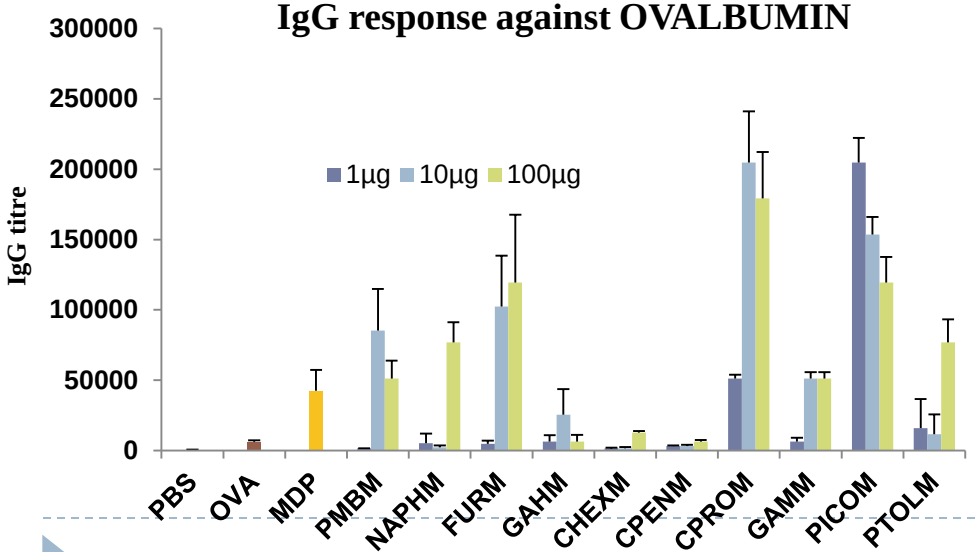
Possible sites of Diversity

# SYNTHESIS OF NOVEL MURAMYL DIPEPTIDE ANALOGUES:
## Immunological Evaluation of Muramyl dipeptide analogues as vaccine adjuvants

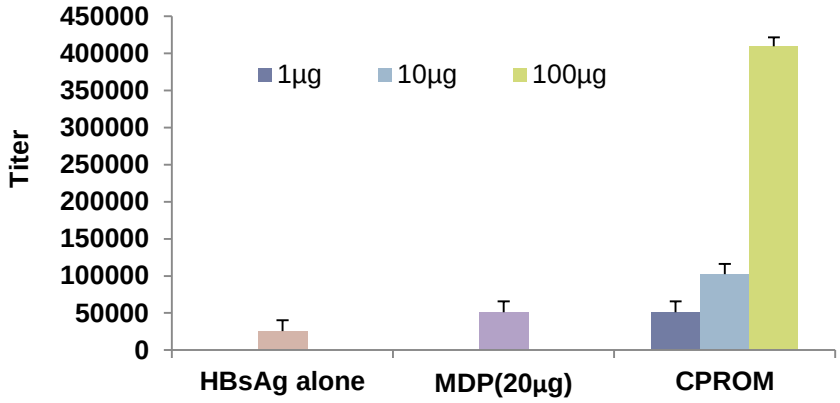



Reagents and conditions:a) BnOH, BF3.Et2O,8 h, 80 oC,90%; b) PhCH(OMe)2,DMF, 7 h, 80 oC,87%; c) KOH, EtOH, 7 h, 130 oC,85%; d) RCOOH, DCC, DMAP, CH2Cl2, 0 oC to rt, 4 h, 96%; e) (S)-(-)-2-Chloropropionic acid, NaH, THF, 65 oC, overnight, 93%; f) 6, EDCI, HOBt, THF, DIPEA, 0 oC to rt, 6 h, 88%; g) H2, Pd/C, AcOH, H2O, rt, 12 h

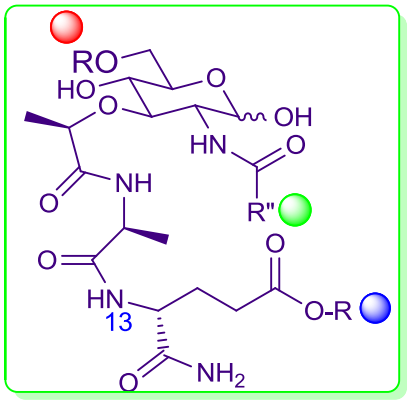
### IgG response against OVALBUMIN

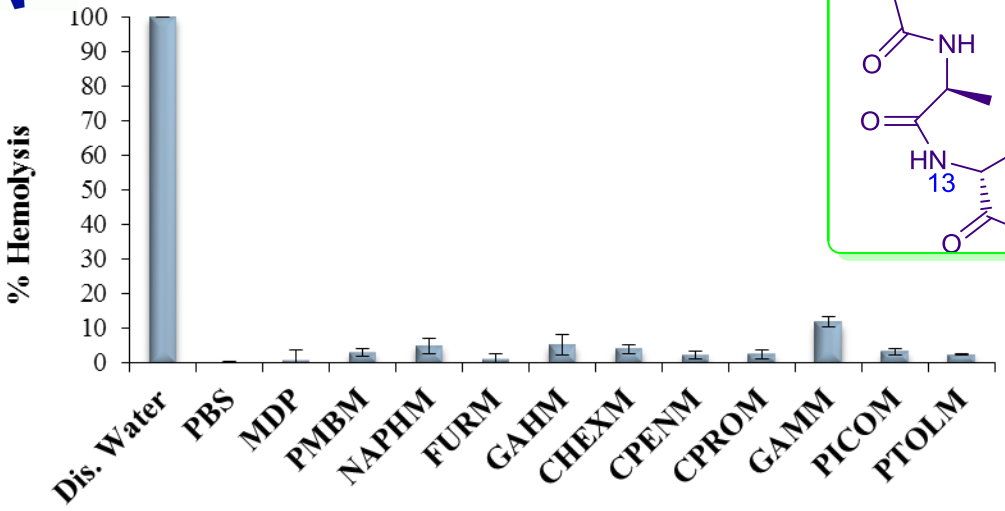


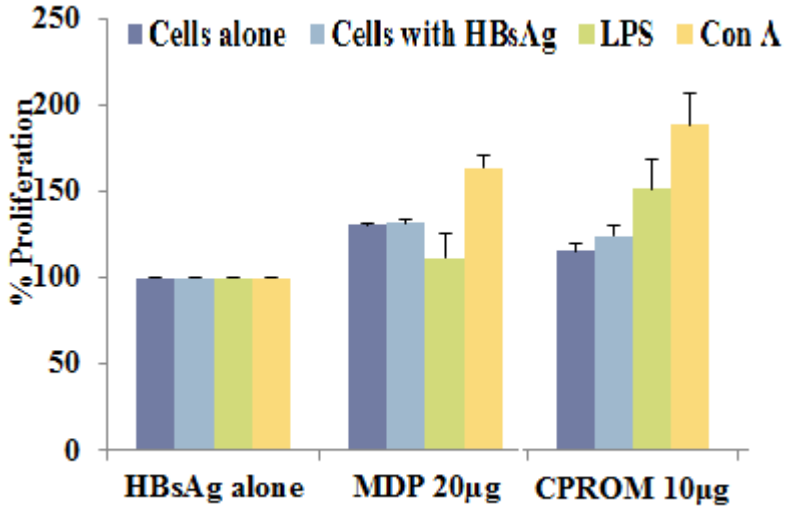

### HBsAg specific antibody response

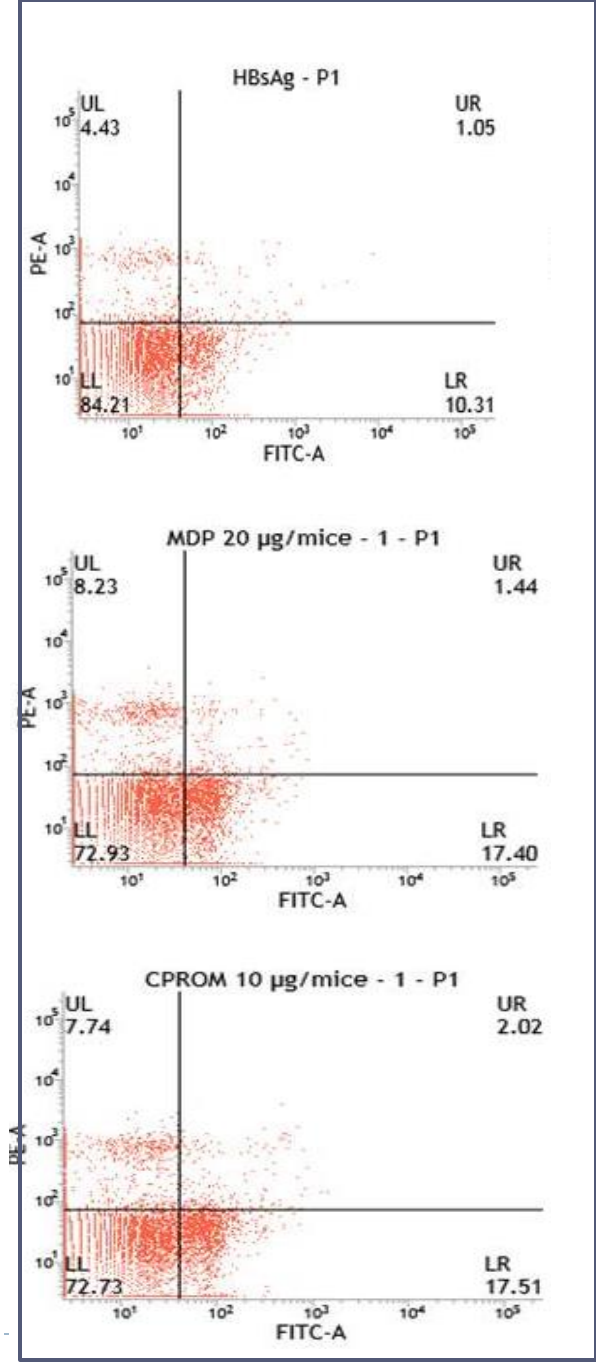





RO
HO
O
OH
HN
O
NH
O
R"
O
HN
13
O
O-R
O
NH2


% Hemolysis
100
90
80
70
60
50
40
30
20
10
0
Dis. Water
PBS
MDP
PMBM
NAPHM
FURM
GAHM
CHEXM
CPENM
CPROM
GAMM
PICOM
PTOLM


250
200
150
100
50
0
% Proliferation
Cells alone
Cells with HBsAg
LPS
Con A
HBsAg alone
MDP 20µg
CPROM 10µg

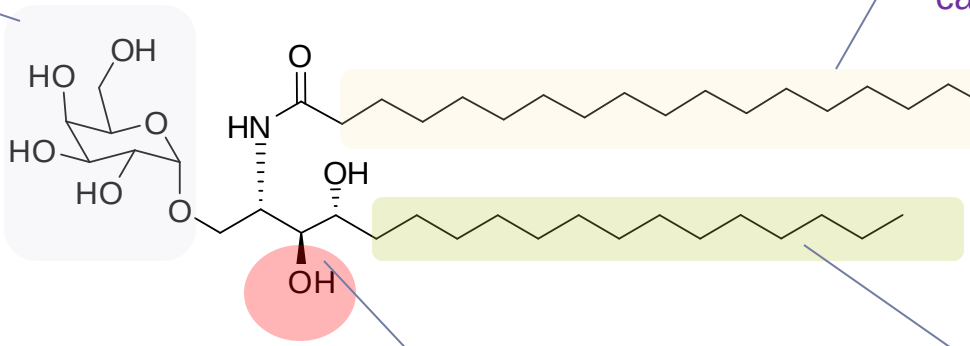

HBsAg - P1
UL
4.43
UR
1.05
LL
84.21
LR
10.31
PE-A
FITC-A
MDP 20 µg/mice - 1 - P1
UL
8.23
UR
1.44
LL
72.93
LR
17.40
PE-A
FITC-A
CPROM 10 µg/mice - 1 - P1
UL
7.74
UR
2.02
LL
72.73
LR
17.51
PE-A
FITC-A

# Structure Activity Relationship(SAR) of α-Galactosylceramide

α-GalCer is a glycolipid antigen derived from extracts of Japanese marine sponge *agelas mauritianus* . It activates *i*-NKT Cells when presented by CD1d and produces large amount of ***cytokines***

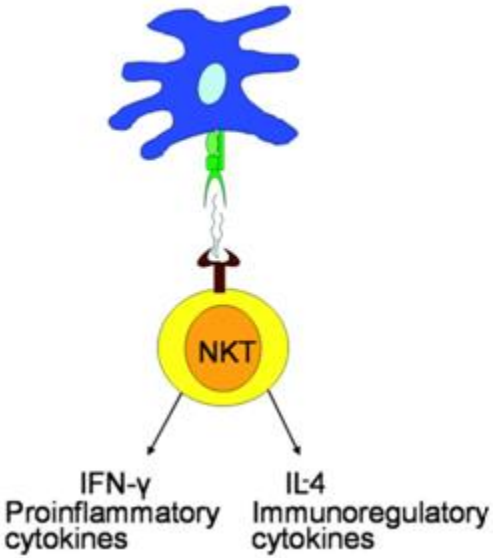
Sugar hydroxyls interact with NKT cell TCR with strong H-bonding network

C26 chain extend into A' pocket of CD1d Which can accommodate up to 80 carbon length



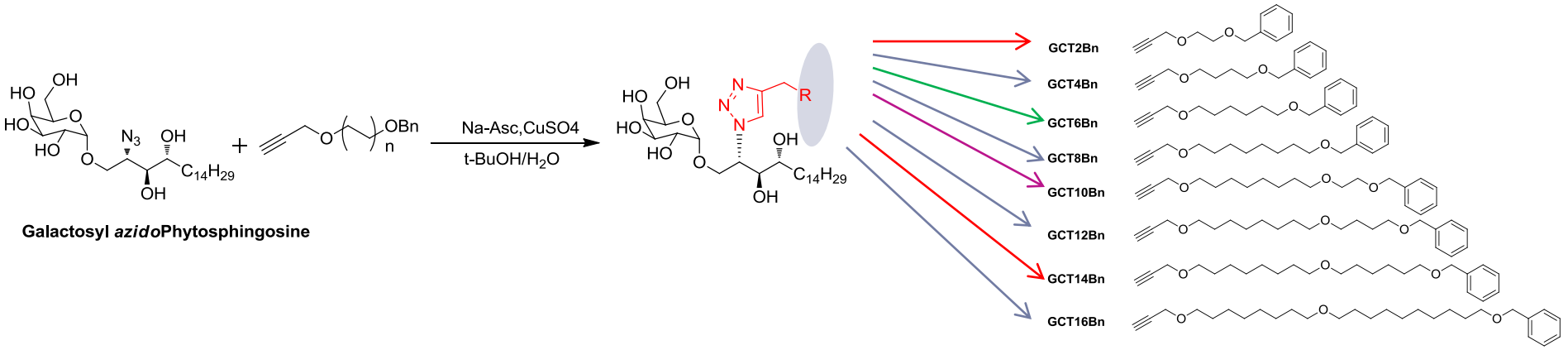

Provide anchor to CD1d /GalCer complex

C14 chain extend into F' pocket of CD1d



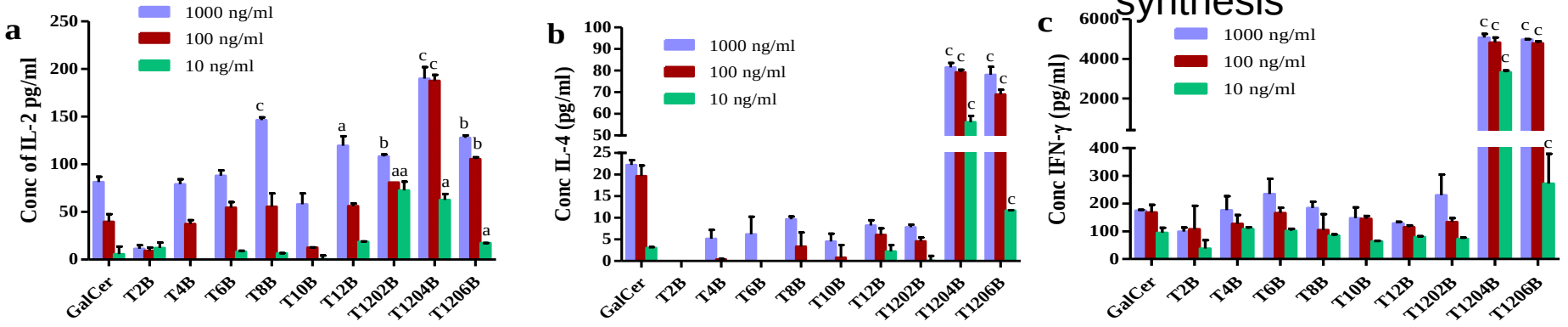



# Immunological Evaluation of α-GalCer Analogues

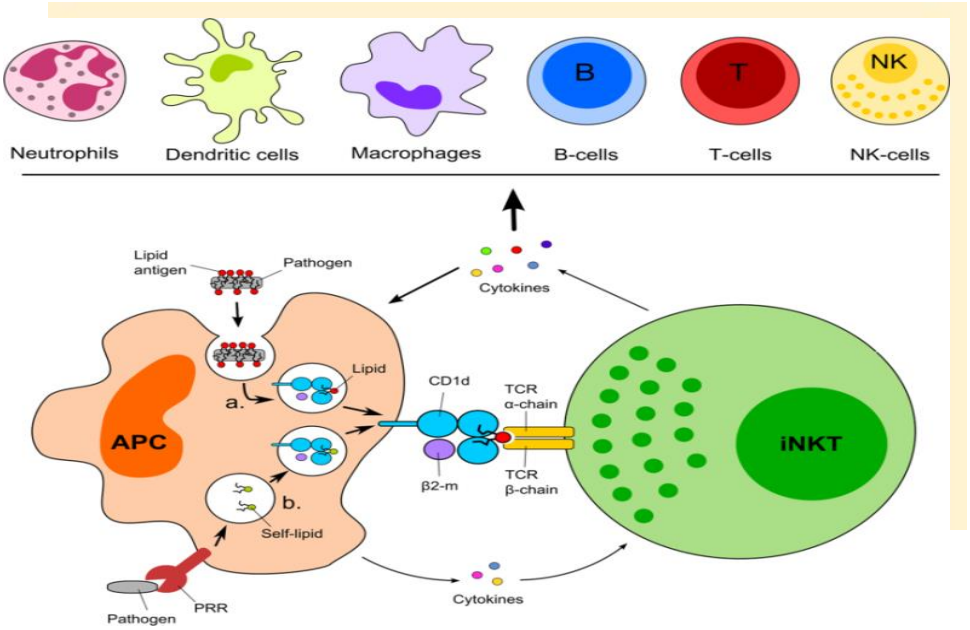



*In vitro* Splenocyte stimulation assay

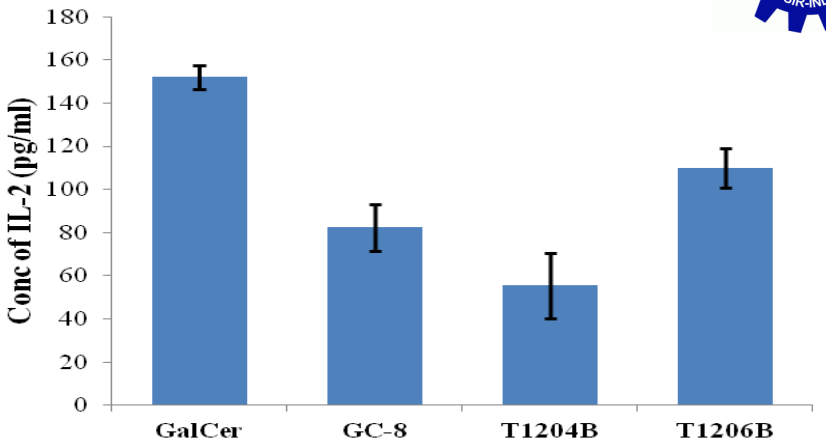
Design and synthesis




Cytokines secretion by mouse splenocytes when stimulated with α-GalCer and its analogues with concentration of 1000, 100 and 10 ng/ml. IL-2, IFN-γ and IL-4 production was measured after 48 hrs treatment.

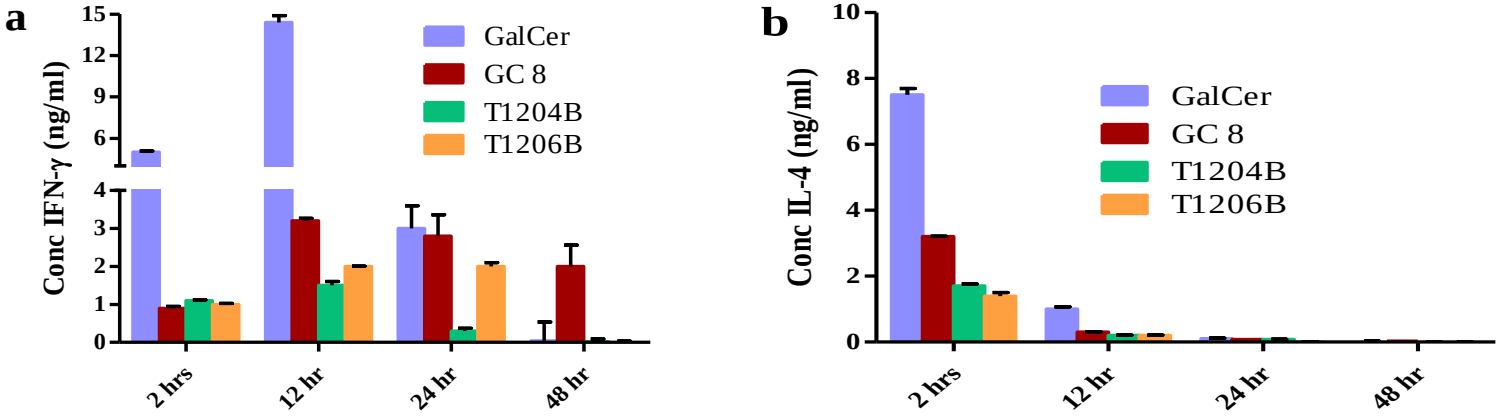



*In vitro* CD1d antigen presentation assay



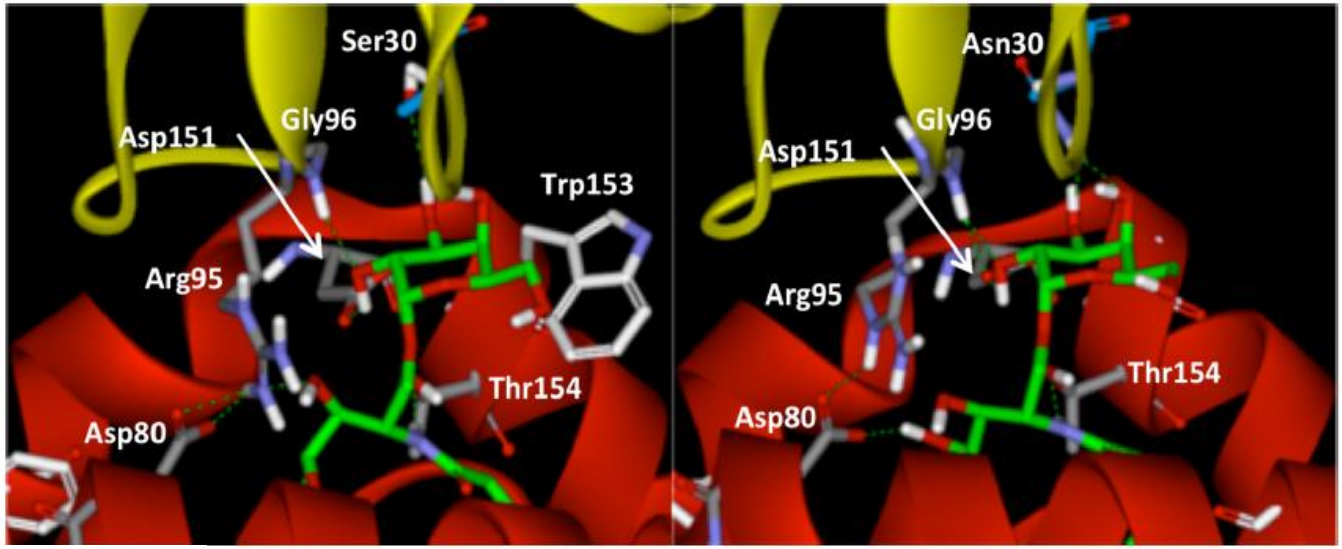

IL-2 expression by *i*NKT hybridoma cells incubated with CD1d protein on treatment with compounds at a concentration of 100 ng/ml for 16 h.

*In vivo* kinetic release of cytokines



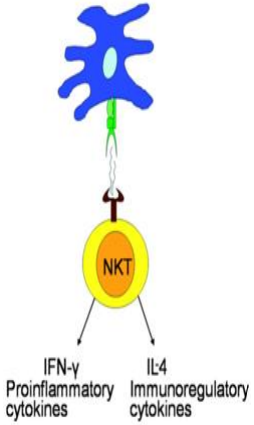

IFN-γ and IL-4 production by BALB/c mice on *in vivo* treatment of compounds at a concentration of 1 µg after 2, 12, 24, and 48 h.

Halmuthur M. et al., *ACS Medicinal Chemistry Letters* (2015, in press )



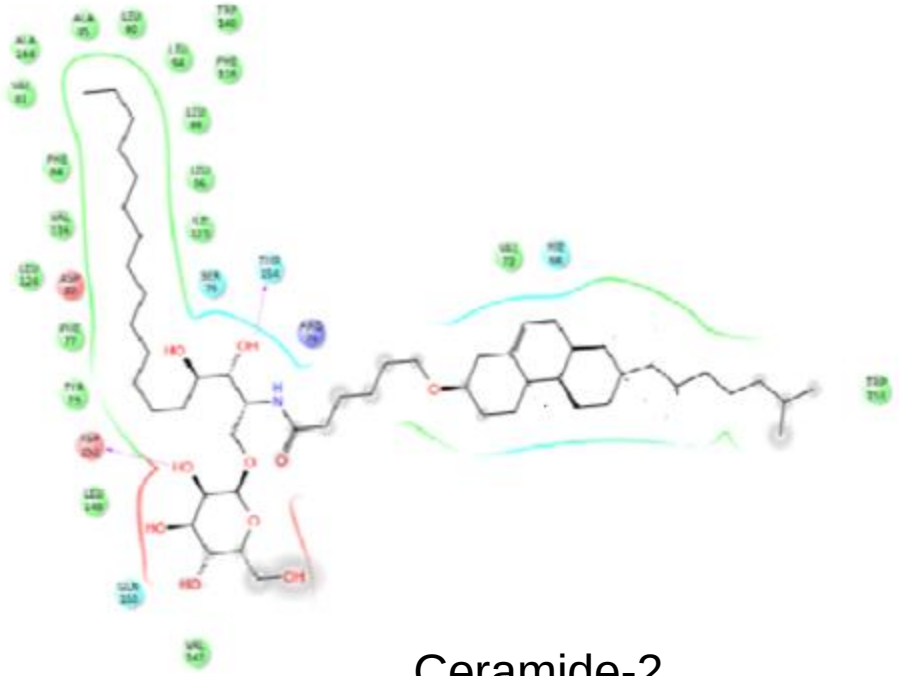

| Ligand | Hydrogen Bond Interaction with CD1d |
|---|---|
| GalCer | Asp-151, Thr-154 |
| Ceramide-1 | Gln-150, Asp-151, Asp-80, Arg-79 |
| Ceramide-2 | Asp-151, Thr-154 |

Philippe Chavatte et al., J. Med. Chem. 2014, 57, 5489−5508



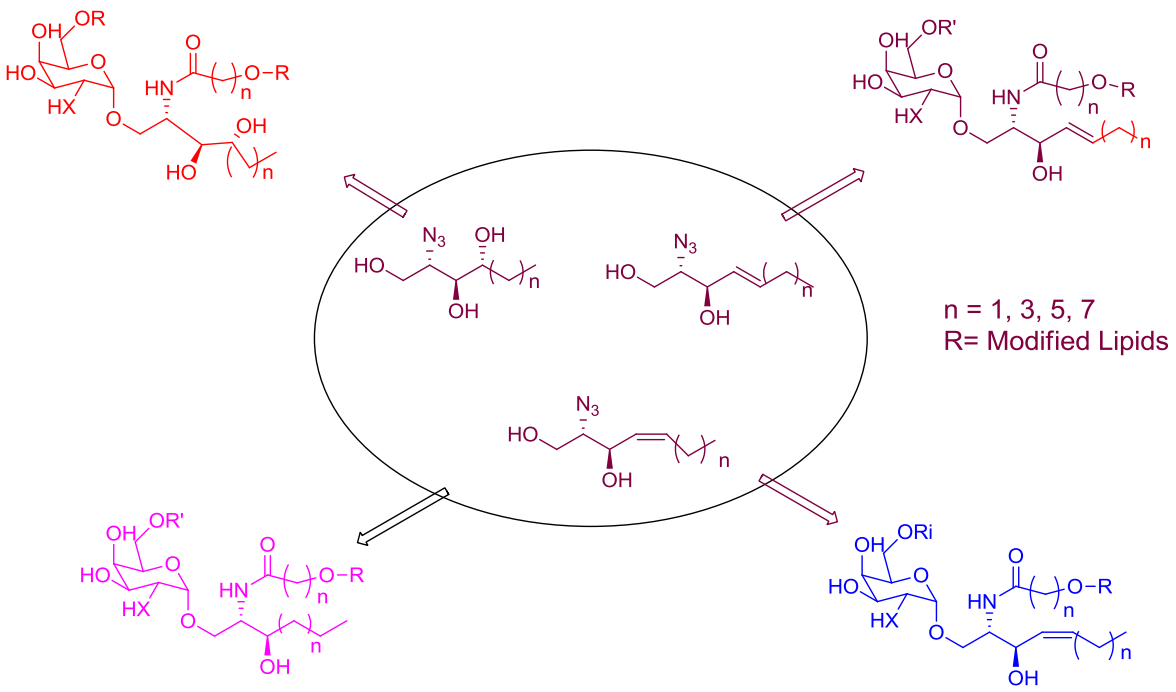



Ceramide-1

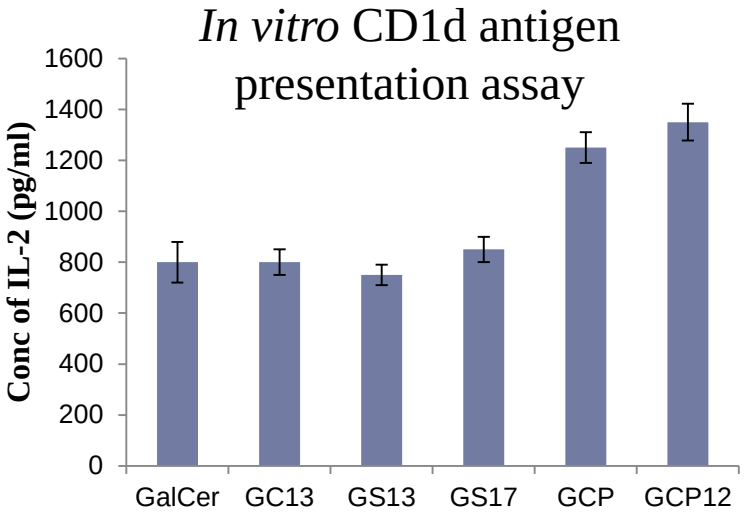


Ceramide-2

# Design and synthesis




**Novel alpha galactosyl ceramide analogues**



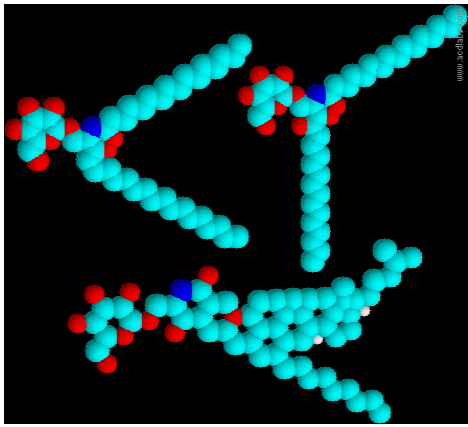
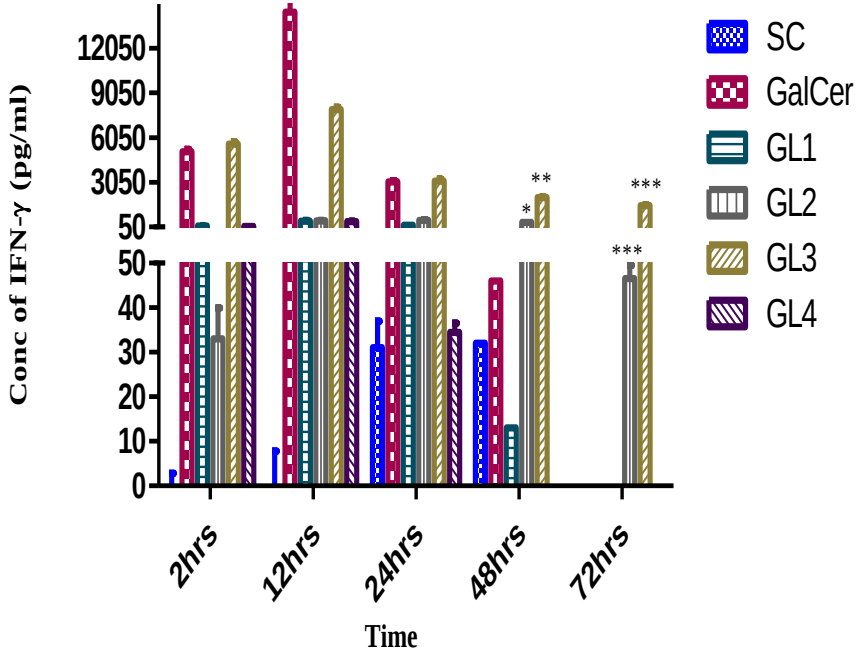
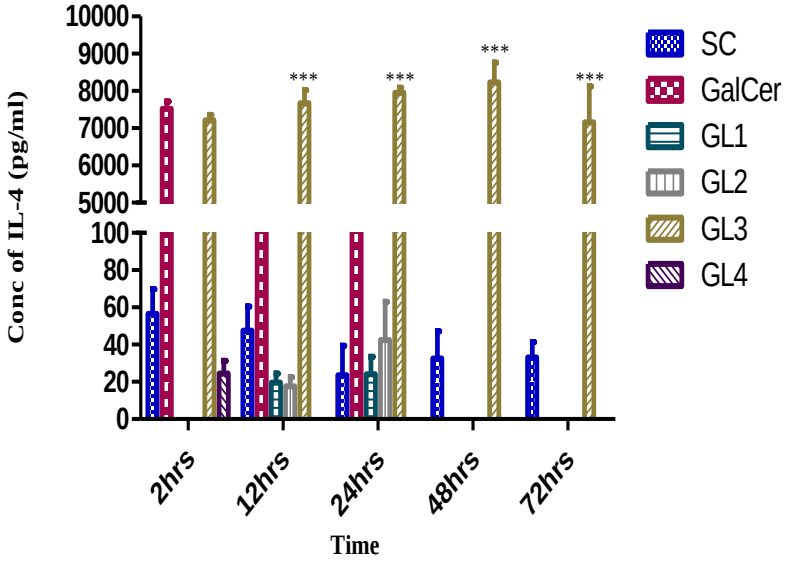
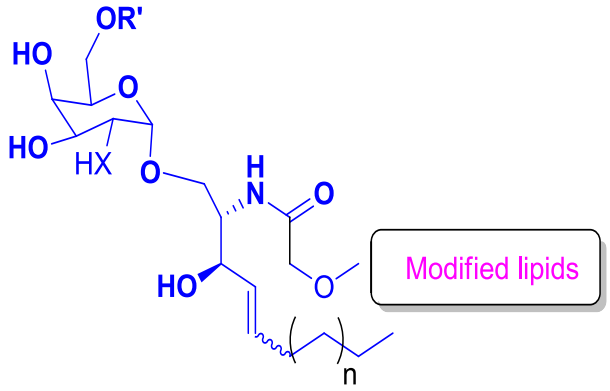



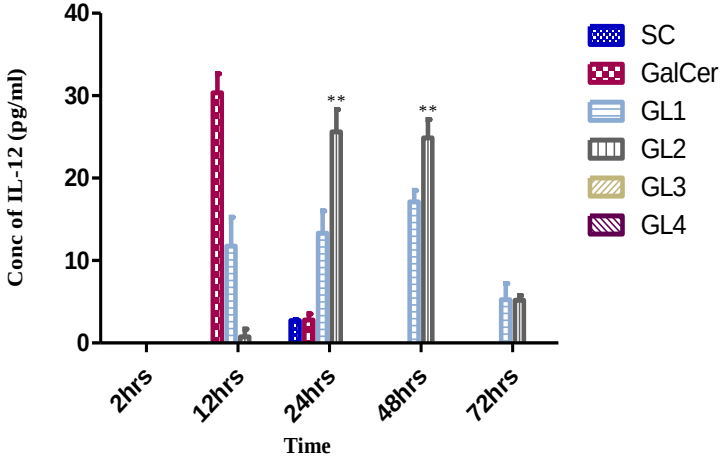
**Fig:** IL-2 secretion by murine hybridomas were stimulated by 1000 ng/ml glycolipids loaded on CD1d coated plates. CD1d molecules (10 µg/ml in PBS) were coated in a 96-well plate by incubation of 1 h at 37°C. IL-2 release was measured after 16 h of culture in a sandwich ELISA.

# *In vivo*-Galactosylceramide analogues– Serum cytokines



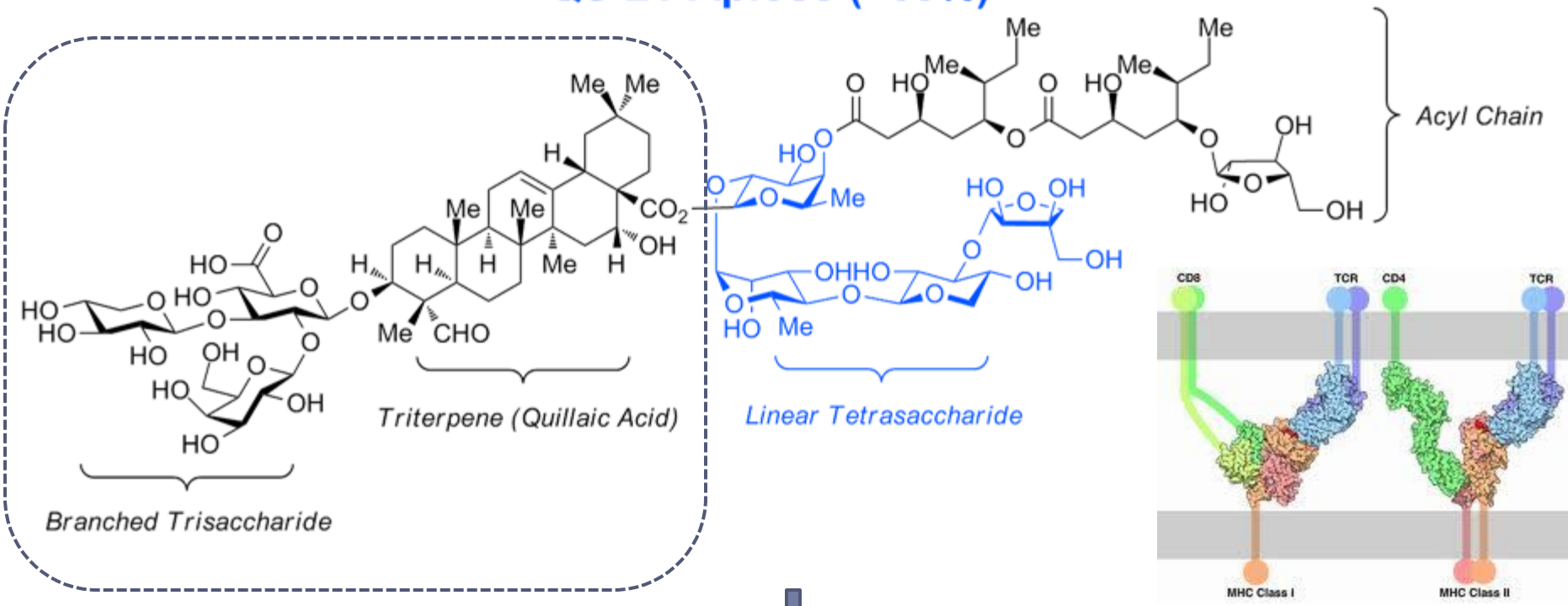




Solvent Control (SC)
PBS containing 0.1% Etoh+0.05% Tween 20

**Kinetics of cytokine release into serum following the injection of glycolipids**. BALB/c male mice were injected intravenously with 1 µg of Glycolipids and then the serum was collected at the indicated times for cytokine profiling.

# Novel saponins as Adjuvants

## QS-21-Apiose (~65%)







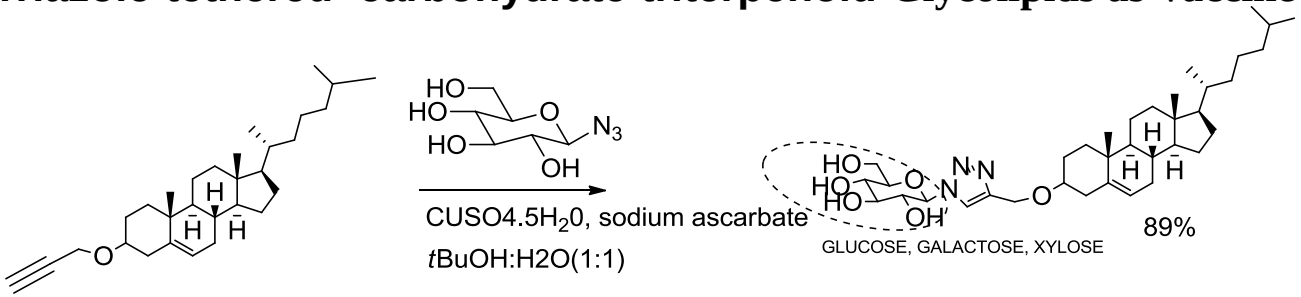
Uniqueness of QS21

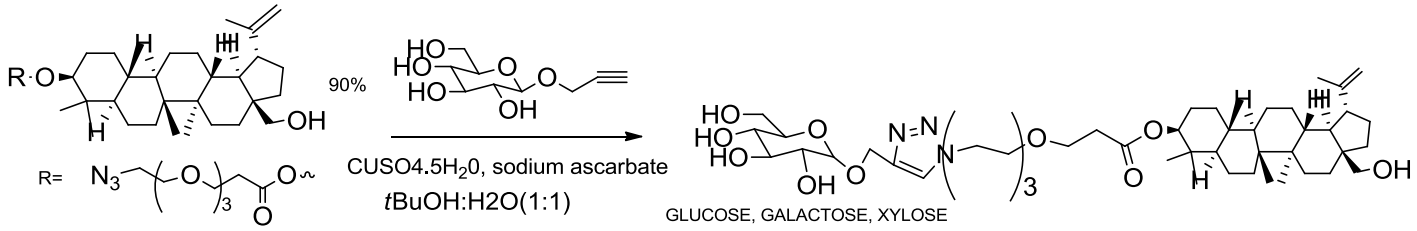
1. Alternating HLB
2. Presence of branched trisaccharide
3. Aldehyde on triterpinoid

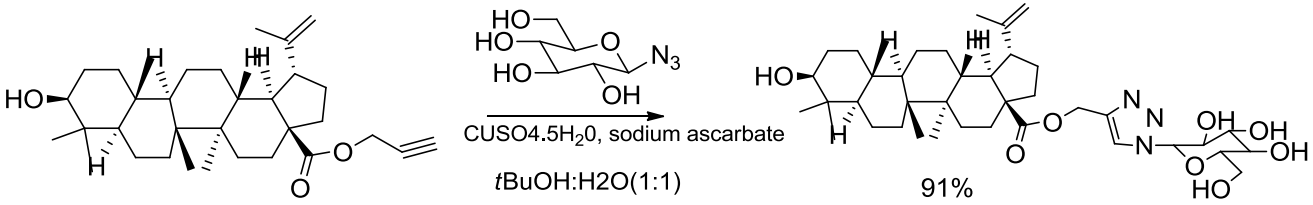
Possibilities

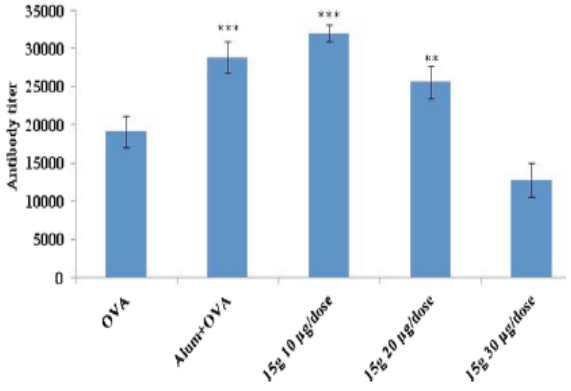
- Simplify the structure
- Introduce formyl group
- Change the triterpinoid

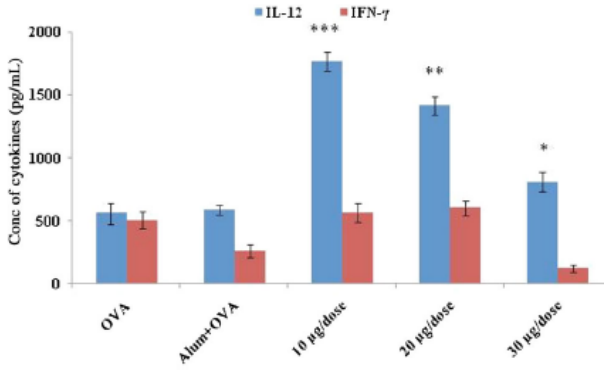
# Design, Synthesis, and Evaluation of 1,2,3-Triazole-tethered carbohydrate triterpenoid Glycolipids as Vaccine Adjuvants

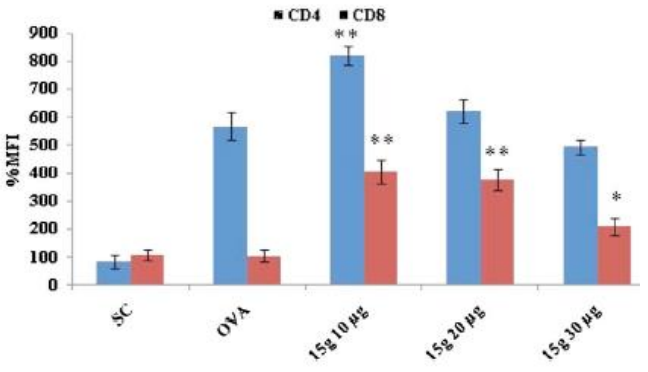


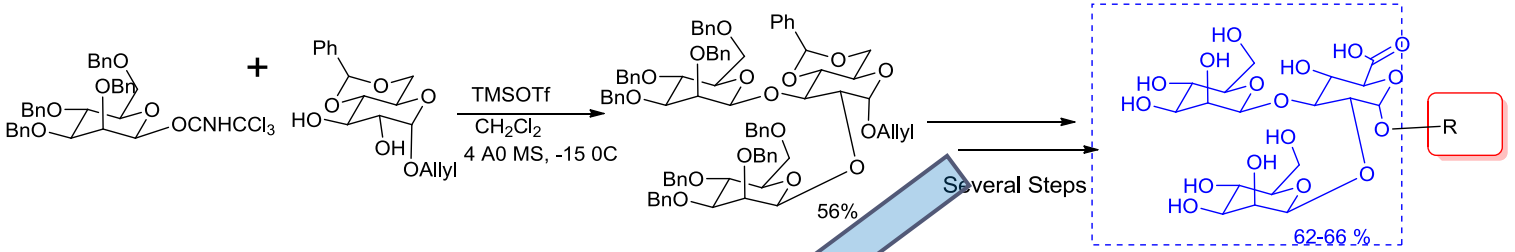



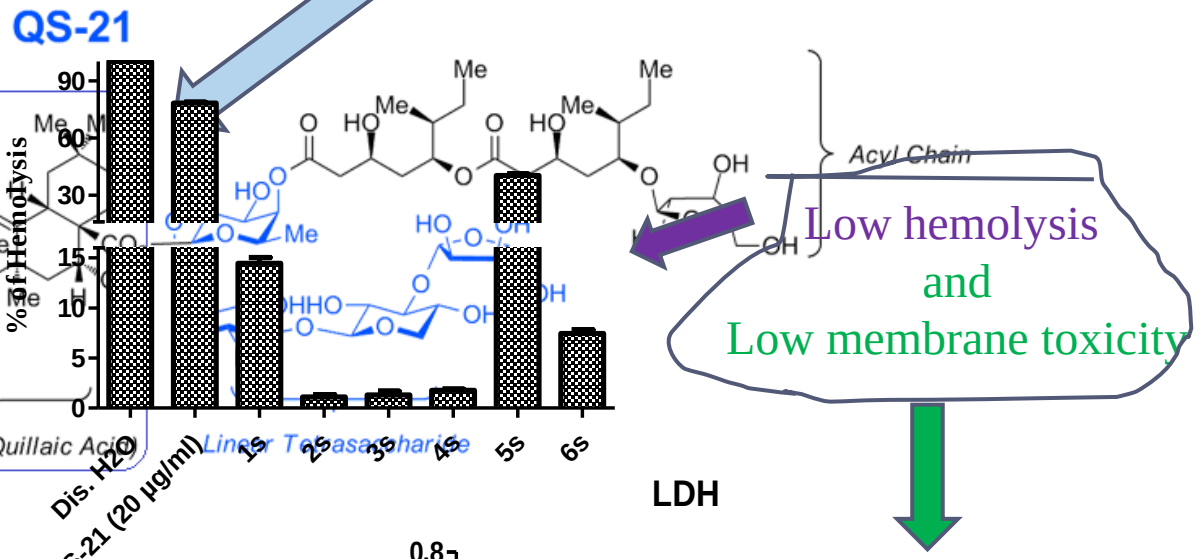



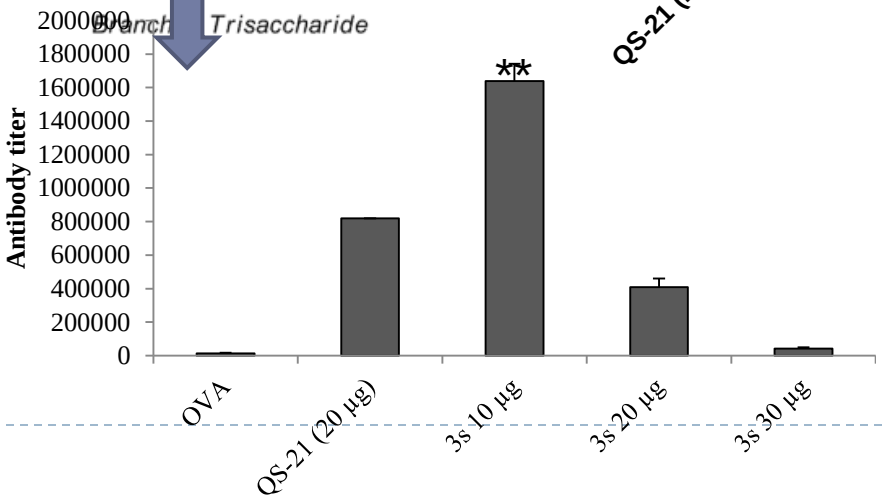

B. Debabrata and Halmuthur Kumar et al., Arch. Pharm. Chem. Life Sci. 2015, 348, 1–15

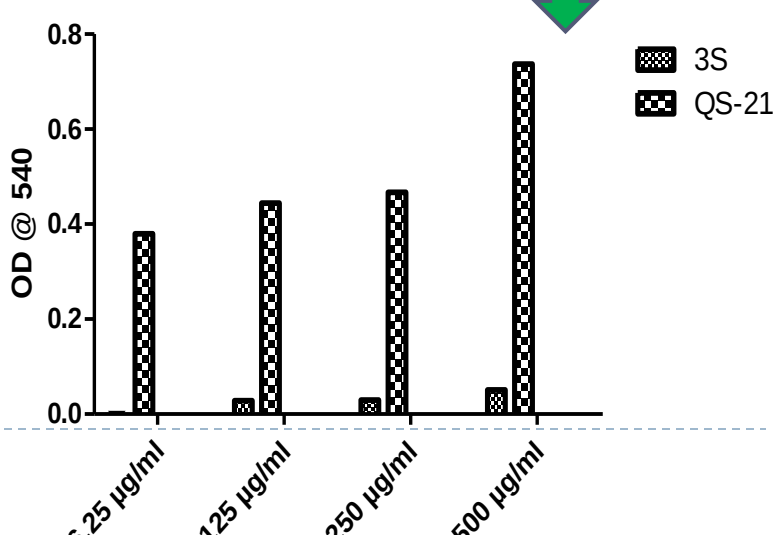
# QS 21 inspired Saponins Mimics as Vaccine Adjuvants




Two fold increase in antibody titer compared to QS-21

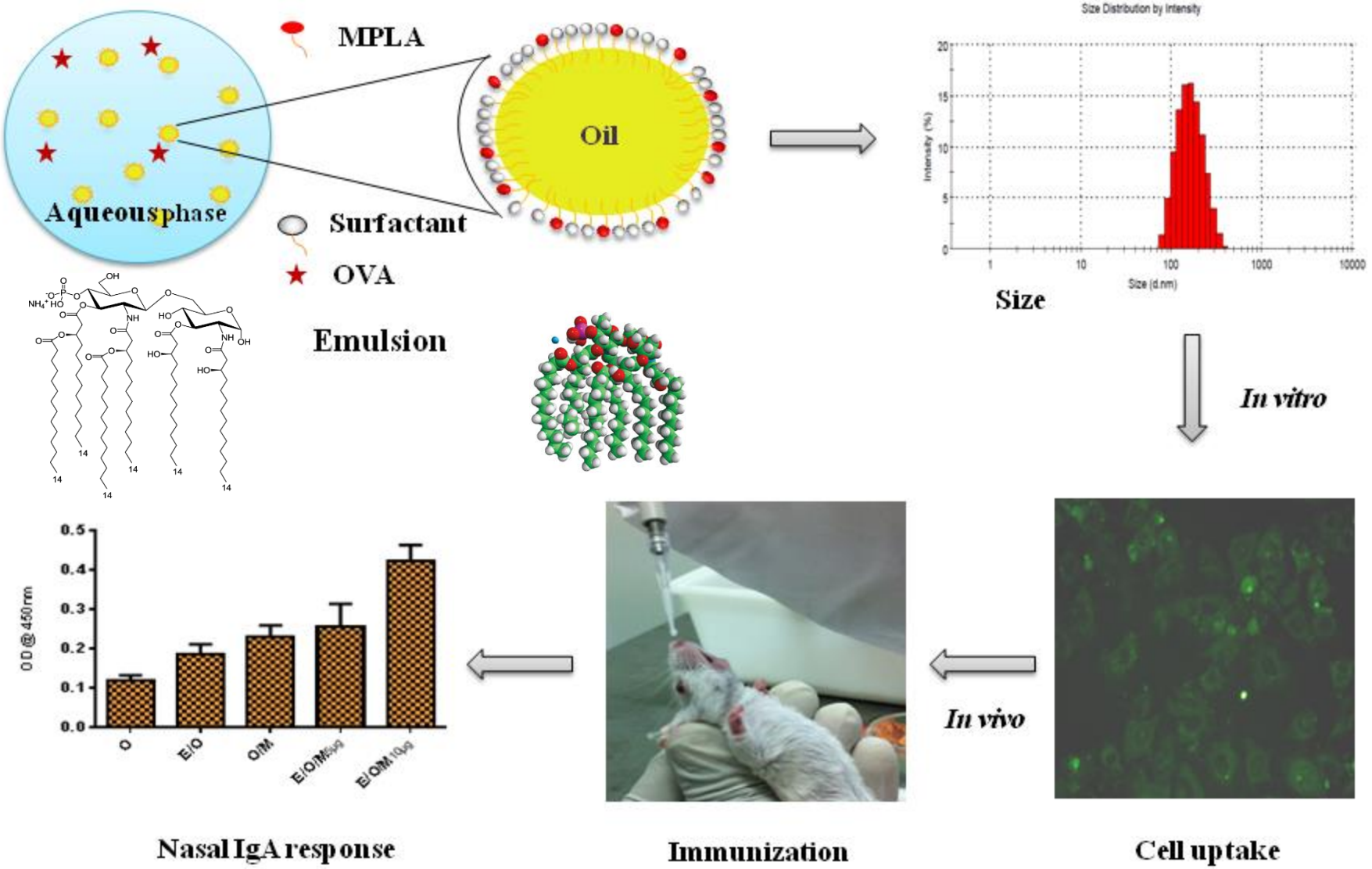


# Docking approach to Adjuvant Design

| TLRs | Active site residues | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| TLR1 | Trp258 | Ile304 | val307 | Ser309 | Asp310 | Val311 | Phe312 | Gly313 | Phe314 | Iso319 | Val333 |
| TLR2 | Asp263 | Arg316 | Iso319 | Arg321 | Phe322 | Tyr323 | Leu324 | Phe325 | Tyr326 | Leu331 | Asn345 |
| TLR3 | Phe314 | Glu363 | Asp366 | Pro368 | Gly309 | Iso310 | Lys311 | Ser312 | Asn313 | Leu318 | Ser392 |
| TLR4 | Tyr255 | leu295 | Val298 | Cys300 | Lys301 | Phe302 | Gly303 | Gln304 | Phe305 | Leu310 | Gly324 |
| TLR5 | Met265 | leu317 | Ala320 | Asn322 | Lys323 | Iso324 | Asn325 | Lys326 | Iso327 | Phe332 | Asn346 |
| TLR6 | Trp263 | iso309 | Iso312 | Asn314 | Gln513 | Val316 | Phe317 | Leu318 | Phe319 | Leu324 | Iso338 |
| TLR7 | Phe408 | leu498 | Ser501 | Asn503 | Ser504 | Iso505 | Phe506 | Phe507 | Val508 | Phe513 | Asn527 |
| TLR8 | Phe423 | leu504 | Ser507 | Asn509 | Ser510 | Iso511 | Phe512 | Phe513 | Iso514 | Phe519 | Asn533 |
| TLR9 | Phe402 | leu477 | Ser480 | Asn482 | Asn483 | Leu484 | Val485 | Tyr486 | Val487 | Phe492 | Asn506 |
| TLR10 | Trp256 | leu304 | val307 | phe309 | arg310 | val311 | phe312 | tyr313 | iso314 | iso319 | iso333 |

# Oleic acid nanoemulsion for nasal vaccination: Impact on adjuvanticity based immune response




Vemireddy Shravanti and Halmuthur Kumar et al., *Journal of Drug Delivery Science and Technology*, 28(2015),






# Vaccine Immunology Laboratory at CSIR-IICT





# SUMMARY

- DC receptor agonists are the best choice for developing adjuvant libraries
- Diversity oriented approach to library generation gives leads to efficacious and inexpensive adjuvants
- Rational approach to design leads to less toxic adjuvants
- Vaccine delivery systems for non invasive administration





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