



# NOVEL CARBOHYDRATE-BASED LIGANDS WITH IMMUNOSTIMULATORY PROPERTIES

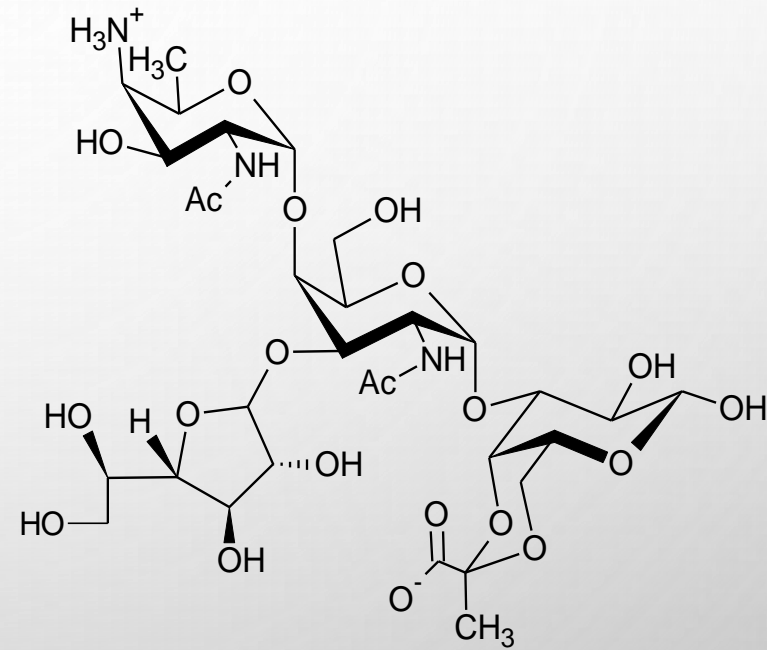
CECILIA H MARZABADI, VIKRAM BASAVA AND CONSTANTINE BITSAKTSIS

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# INTRODUCTION

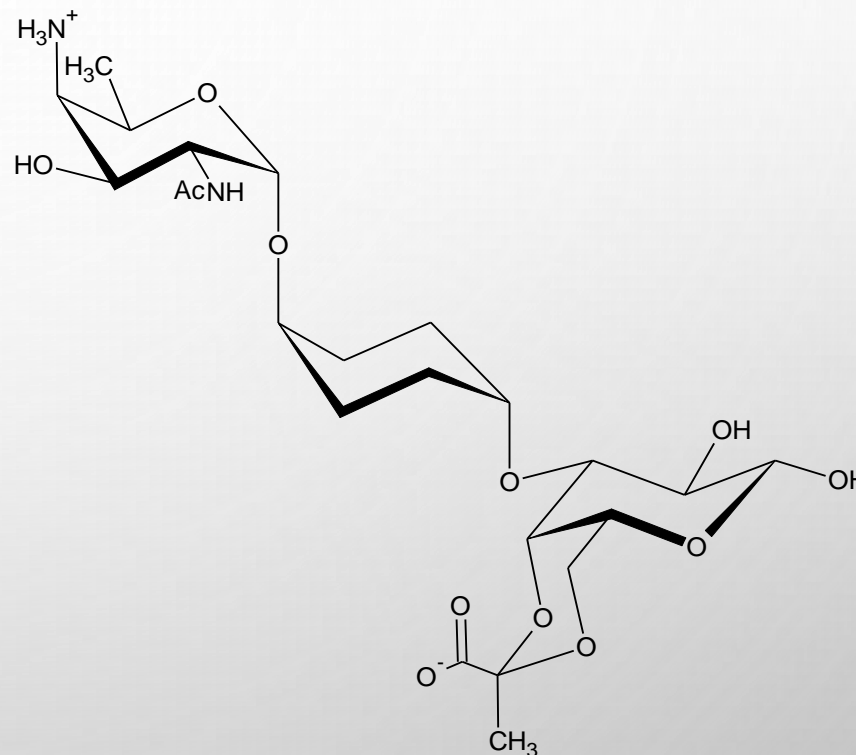
- THE ZWITTERIONIC POLYSACCHARIDE, PSA1, ISOLATED FROM THE CAPSULE OF *BACTEROIDES FRAGILIS*, HAS BEEN SHOWN TO STIMULATE BOTH INNATE AND ADAPTIVE IMMUNE RESPONSES IN MAMMALS AND IS BELIEVED TO BE IMPORTANT IN ESTABLISHING A BALANCED IMMUNE SYSTEM WHICH IS MEDIATED BY T-LYMPHOCYTES.



1

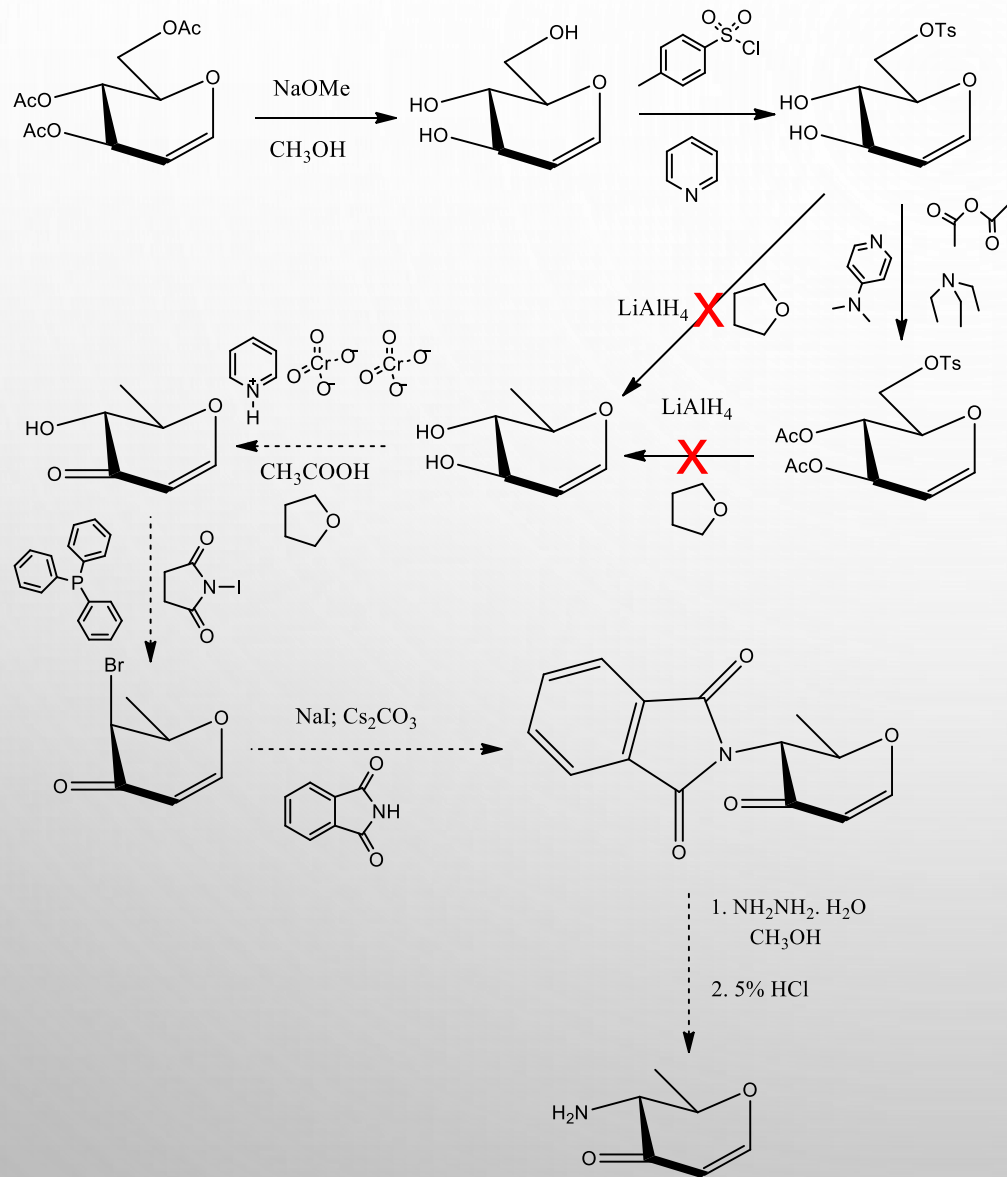
# INTRODUCTION

- WORK IN OUR LABORATORY INVOLVES THE SYNTHESIS OF SIMPLER ZWITTERIONIC SACCHARIDE ANALOGUES FOR EVALUATION AS POTENTIAL STIMULANTS OF INNATE AND/OR ADAPTIVE IMMUNITY.
- RECENTLY, ONE SUCH ANALOG, **2**, HAS BEEN SYNTHESIZED AND HAS BEEN SHOWN TO POSSESS IMMUNOSTIMULATORY PROPERTIES.



**2**

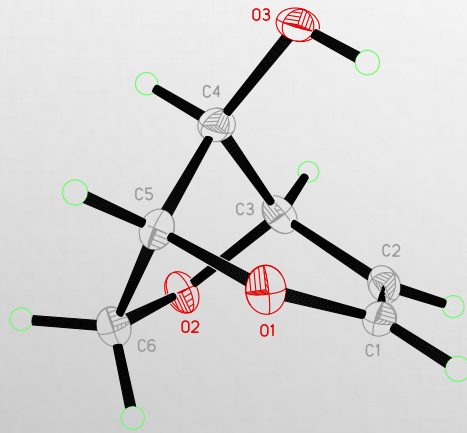
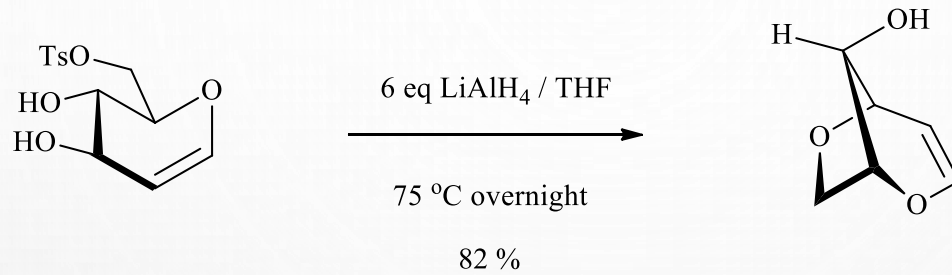
**3**



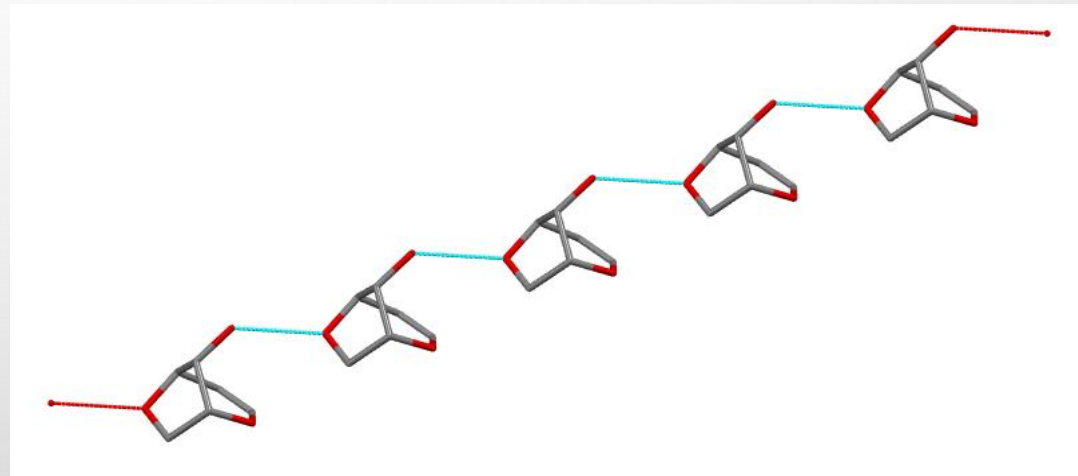
# SYNTHETIC STUDIES

## Synthesis of Subunit A: (Proposed Scheme)

# SYNTHETIC STUDIES



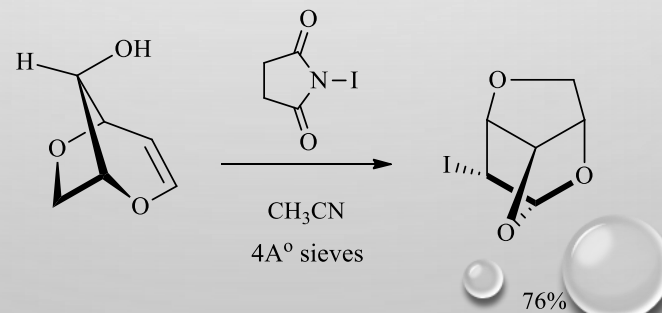
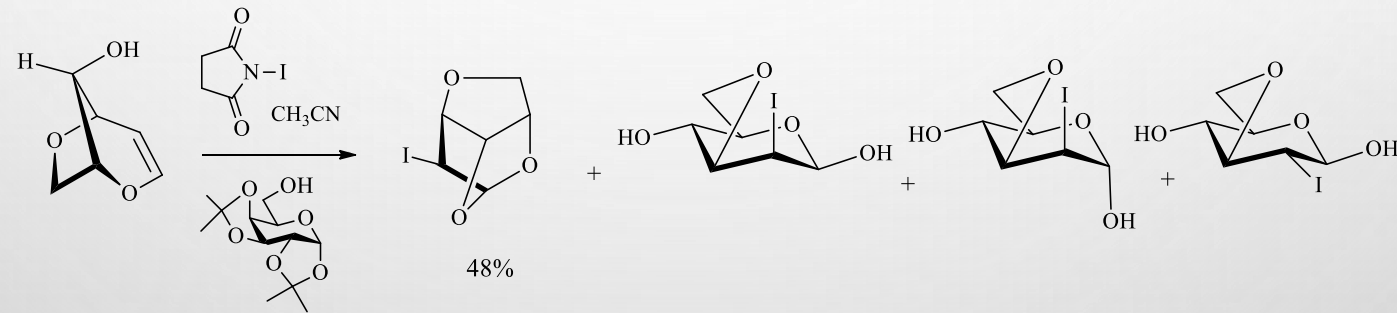
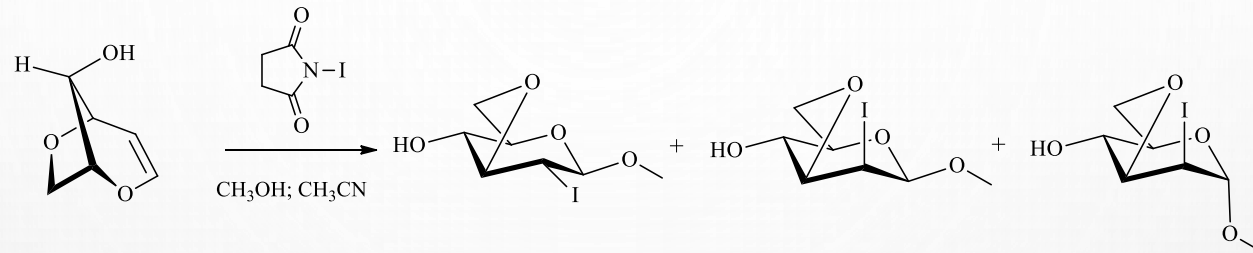
ORTEP representation  
of the anhydride



3,6-Anhydride molecules showing intermolecular H-bonding

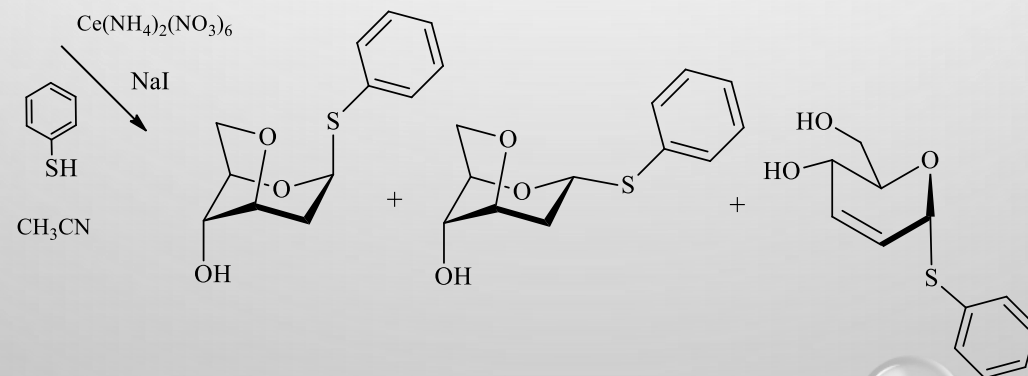
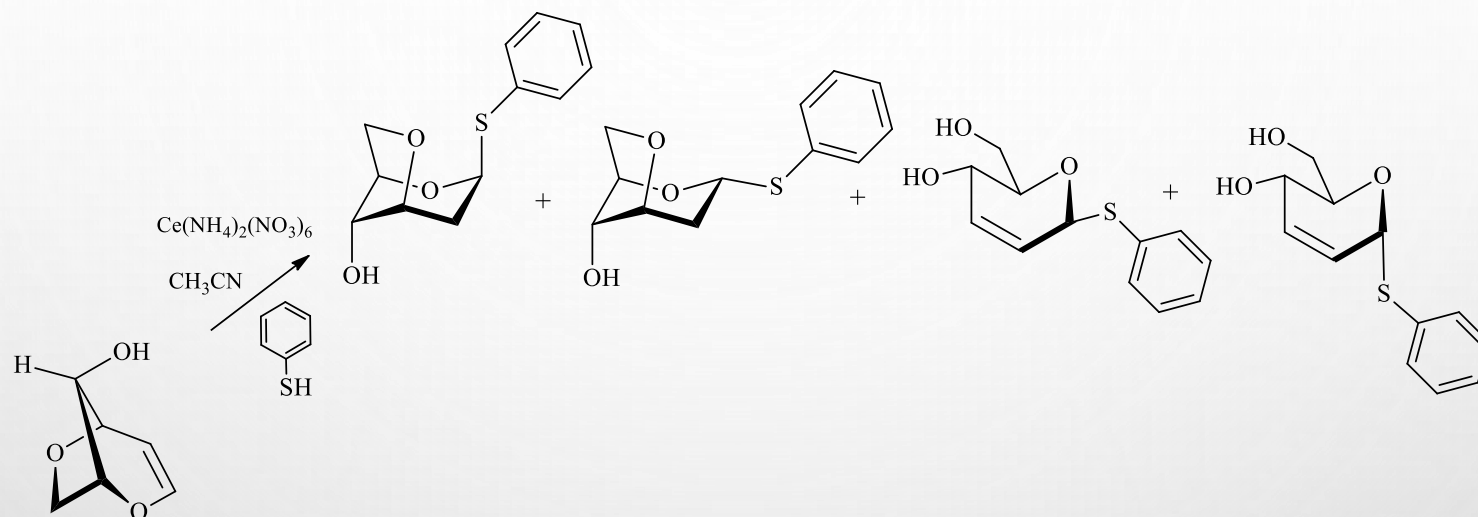
# SYNTHETIC STUDIES

## Reactivity of 3,6-Anhydro-D-glucal:



# SYNTHETIC STUDIES

## Reactivity of 3,6-Anhydro-D-glucal:







## Carbohydrate Research

Available online 13 March 2014

In Press, Accepted Manuscript — Note to users



### **An improved synthesis of 3,6-anhydro-D-glucal and a study of its unusual chemical reactivity**

Vikram Basava, Sergiu M. Gorun, Cecilia H. Marzabadi  

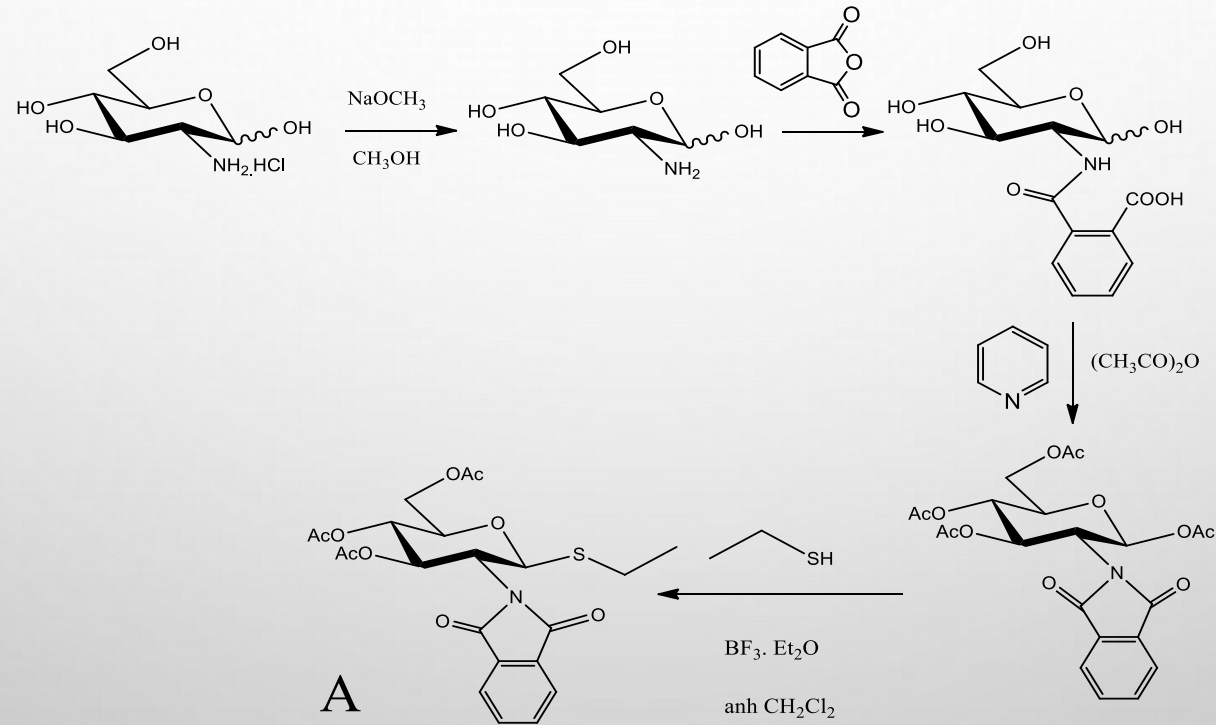
Department of Chemistry & Biochemistry, Seton Hall University, 400 South Orange Ave, South Orange, NJ, USA

Received 5 January 2014, Revised 28 February 2014, Accepted 5 March 2014, Available online 13 March 2014



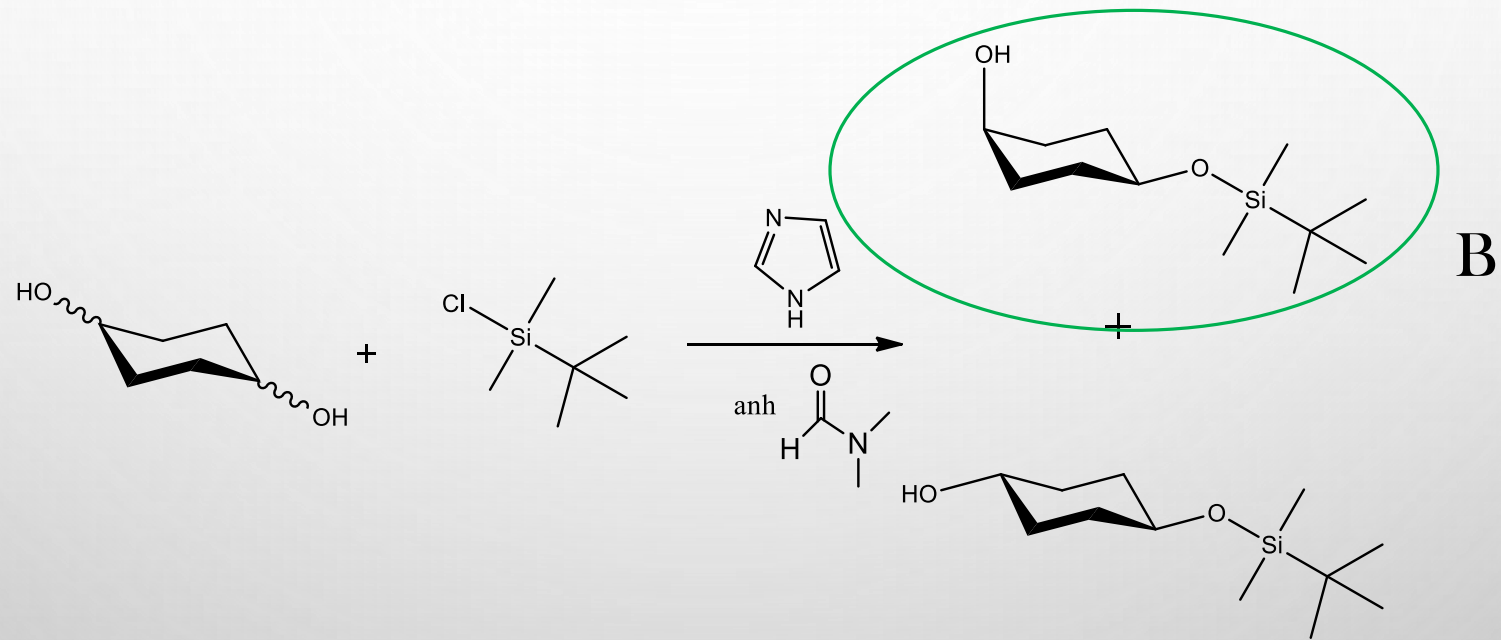
# SYNTHETIC STUDIES

## Synthesis of Subunit A:



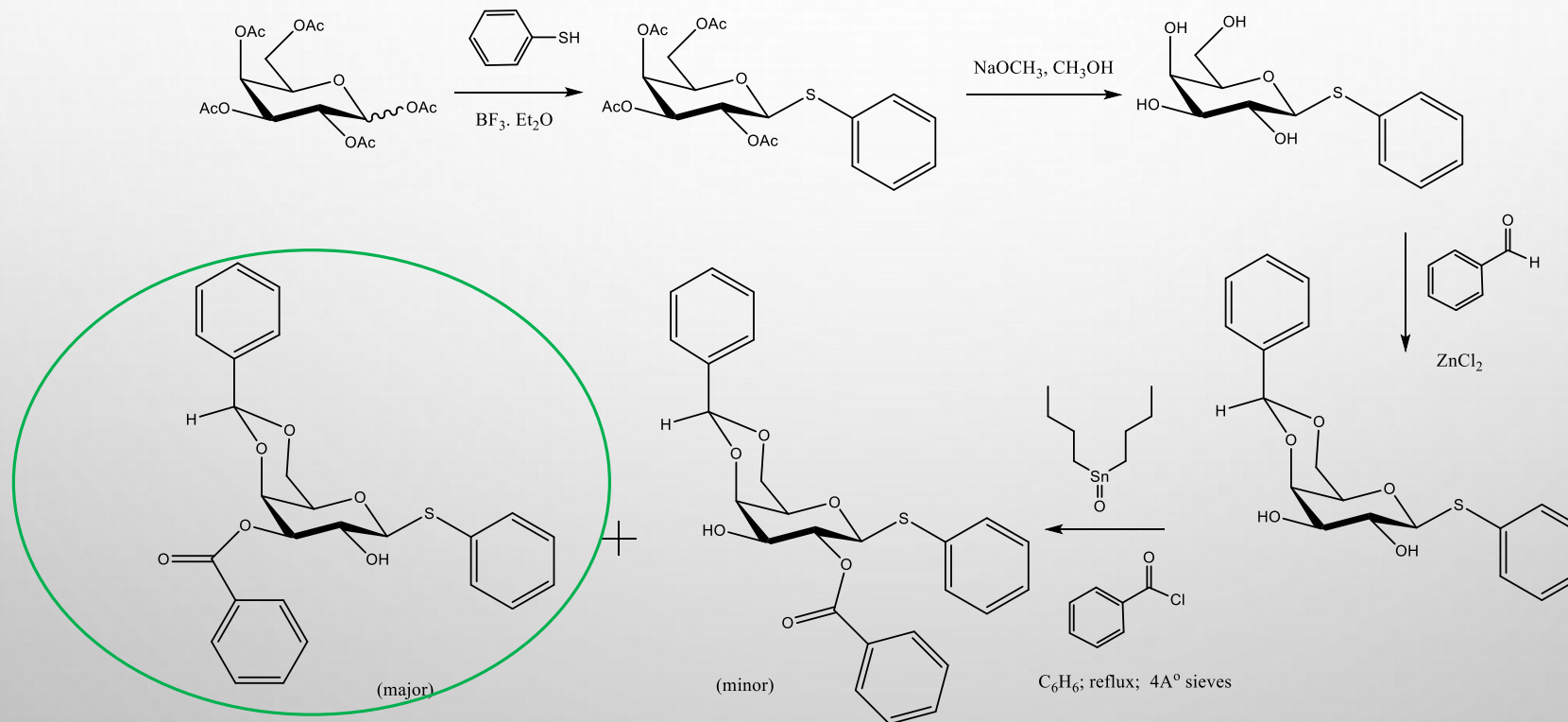
# SYNTHETIC STUDIES

## Synthesis of Subunit B:



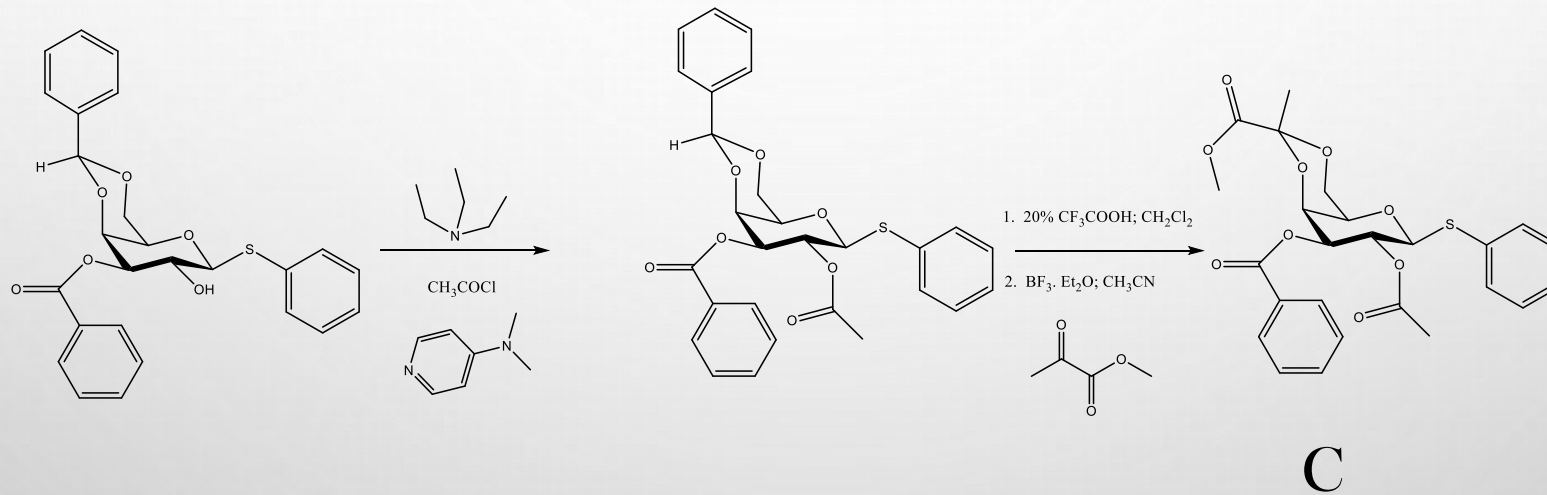
# SYNTHETIC STUDIES

## Synthesis of Subunit C:



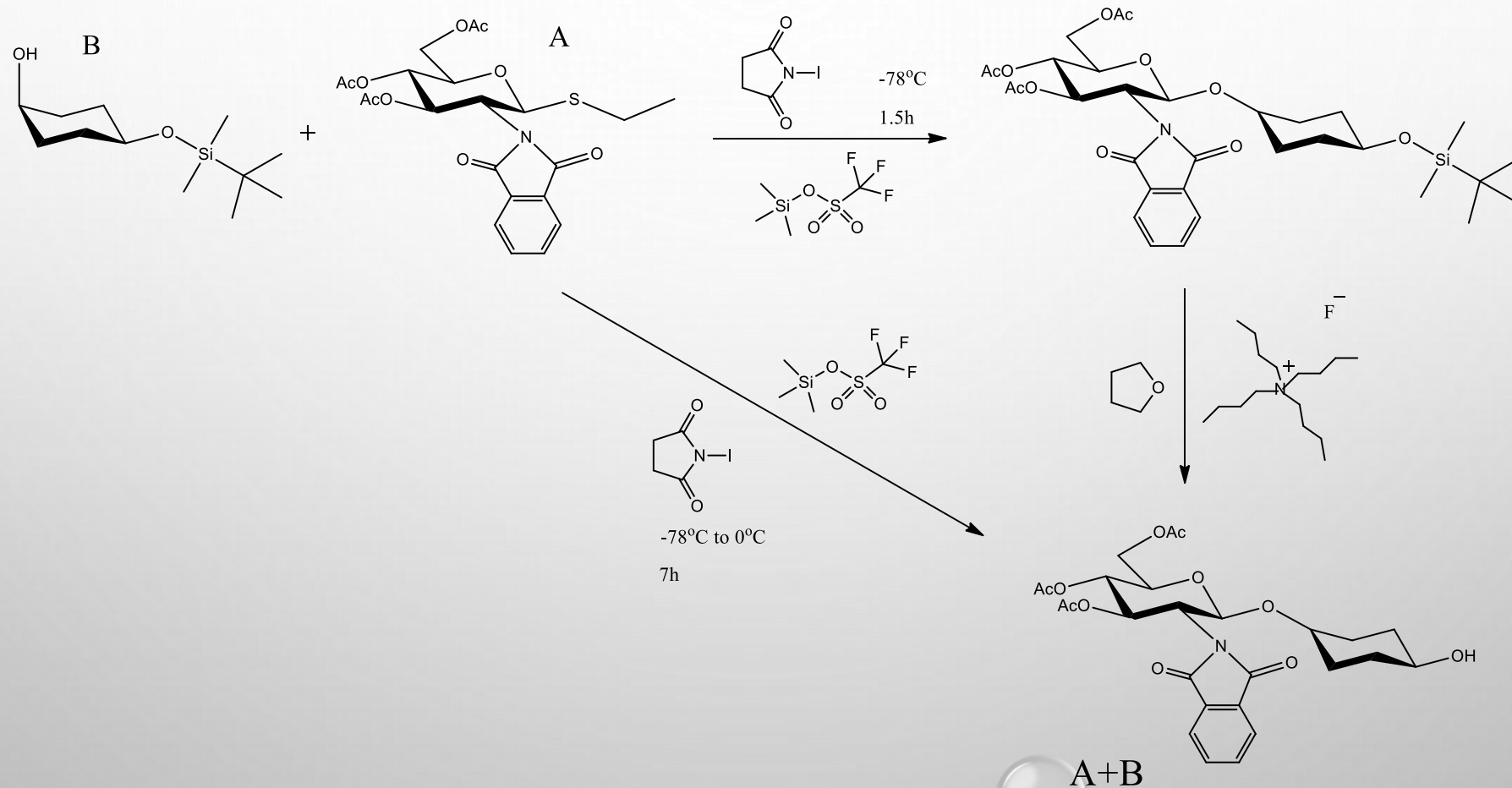
# SYNTHETIC STUDIES

## Synthesis of Subunit C (cont):



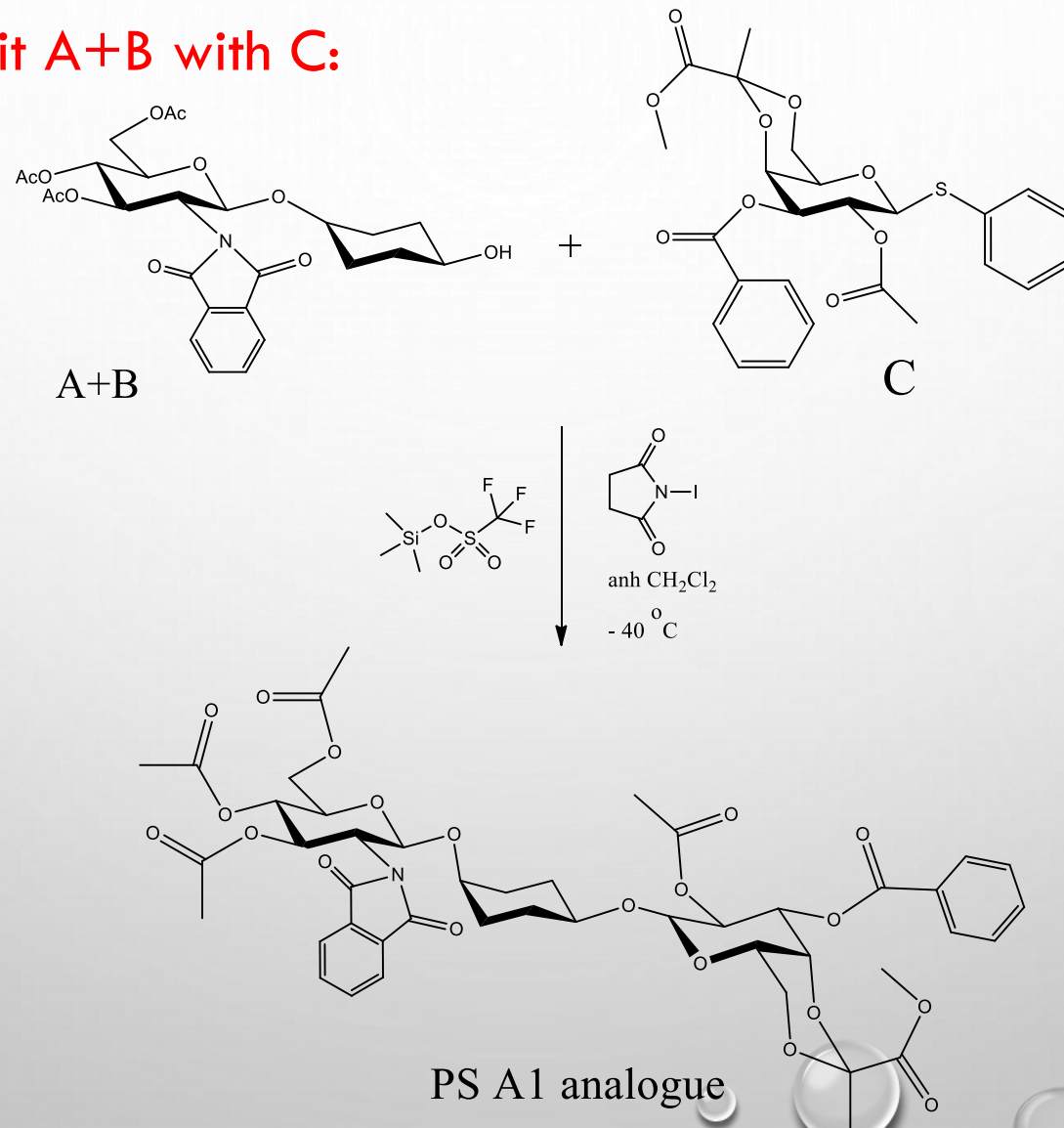
# SYNTHETIC STUDIES

## Coupling of Subunits A and B:



# SYNTHETIC STUDIES

## Coupling of Subunit A+B with C:



# BIOLOGICAL EVALUATION

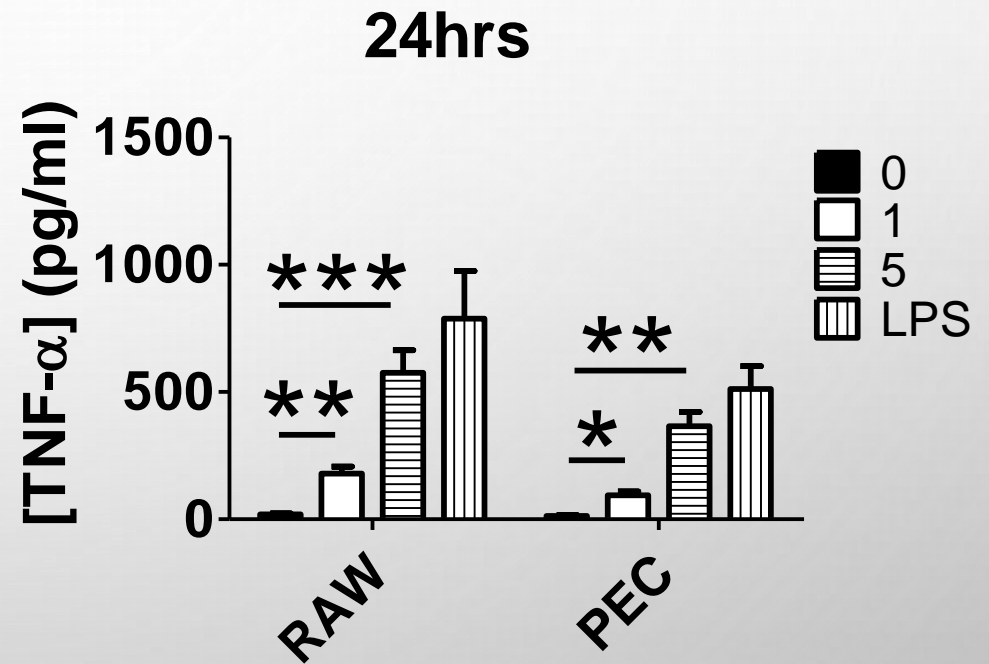
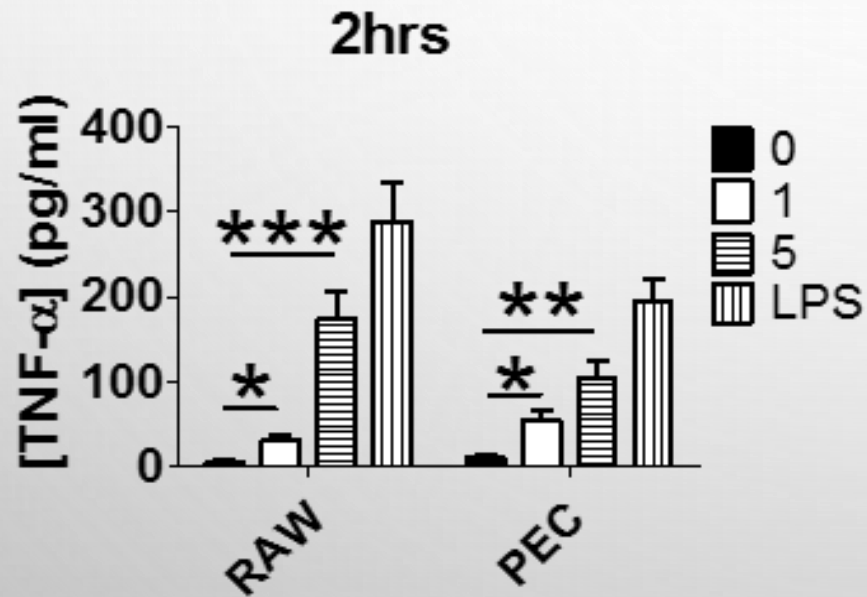
## Innate Immunity:

- PROTECTED ANALOG 2 WAS EVALUATED IN ELISA ASSAYS FOR THE PRODUCTION OF THE PROINFLAMMATORY CYTOKINES TNF- $\alpha$  AND IL-6 USING RAW 264.7 AND MOUSE PERITONEAL MACROPHAGES (C57 BL/6, JACKSON)

# BIOLOGICAL EVALUATION

## Innate Immunity:

TNF- $\alpha$

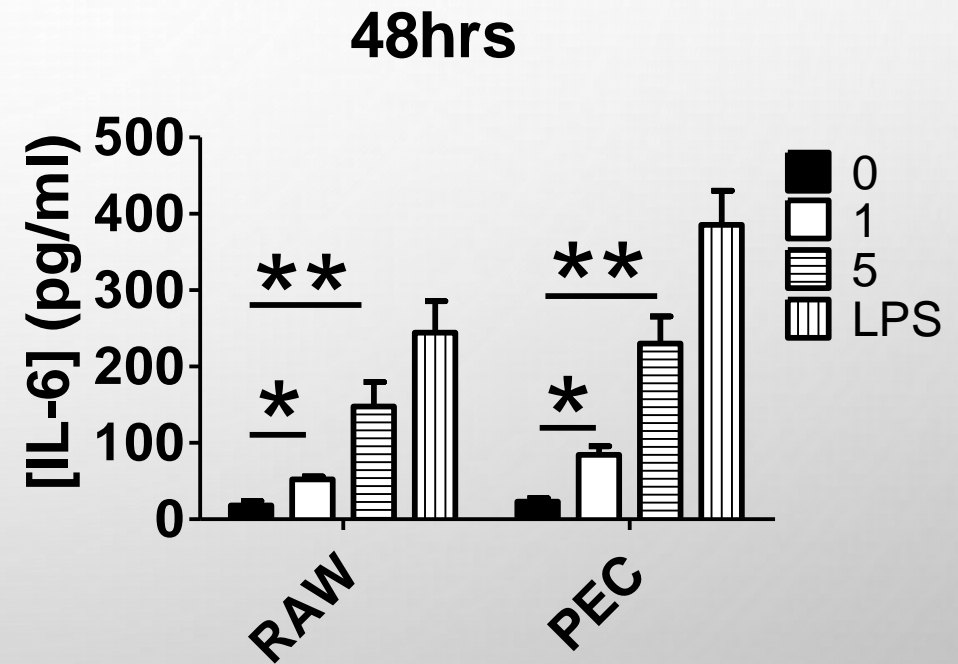
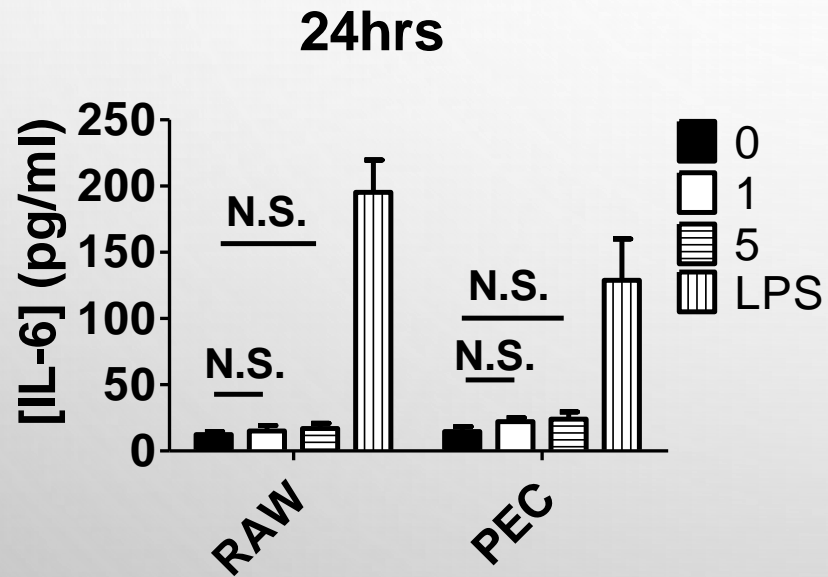




# BIOLOGICAL EVALUATION

## Innate Immunity:

IL-6



# BIOLOGICAL EVALUATION

## Adaptive Immunity:

- THE MOLECULE SYNTHESIZED WAS ALSO TESTED IN SPLEEN CELLS FROM THE SAME MICE LOOKING FOR T AND B CELL PROLIFERATION, LEVELS OF IFN-G IN THE SUPERNATANTS (INDICATIVE OF A T CELL RESPONSE), AND POLYCLONAL IGM AND IGG LEVELS IN THE SUPERNATANTS L (INDICATIVE OF A B CELL RESPONSE) WERE MEASURED.
- NO T CELL OR B CELL RESPONSES WERE OBSERVED.

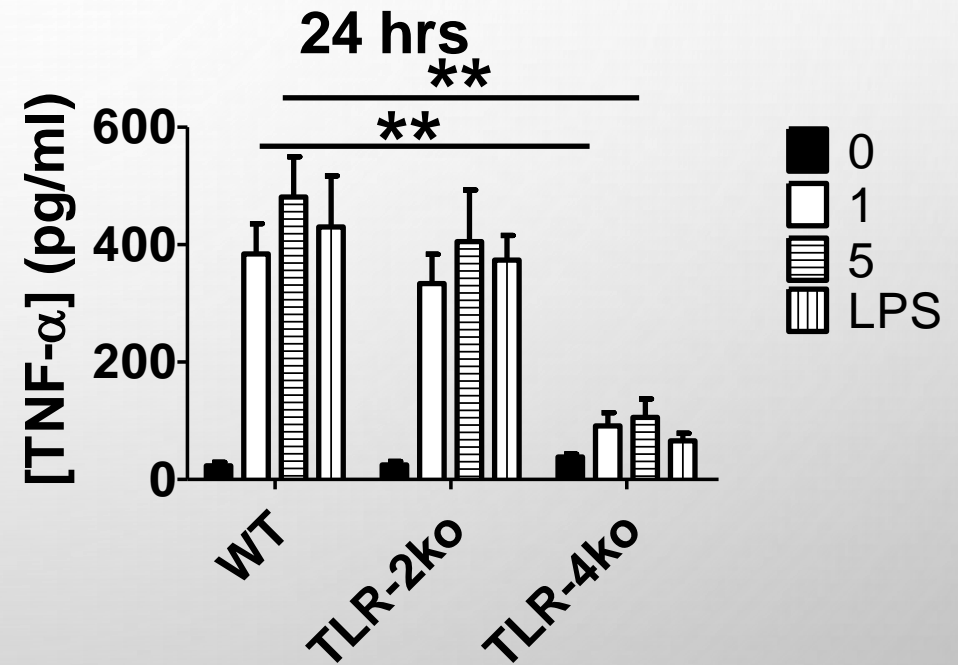
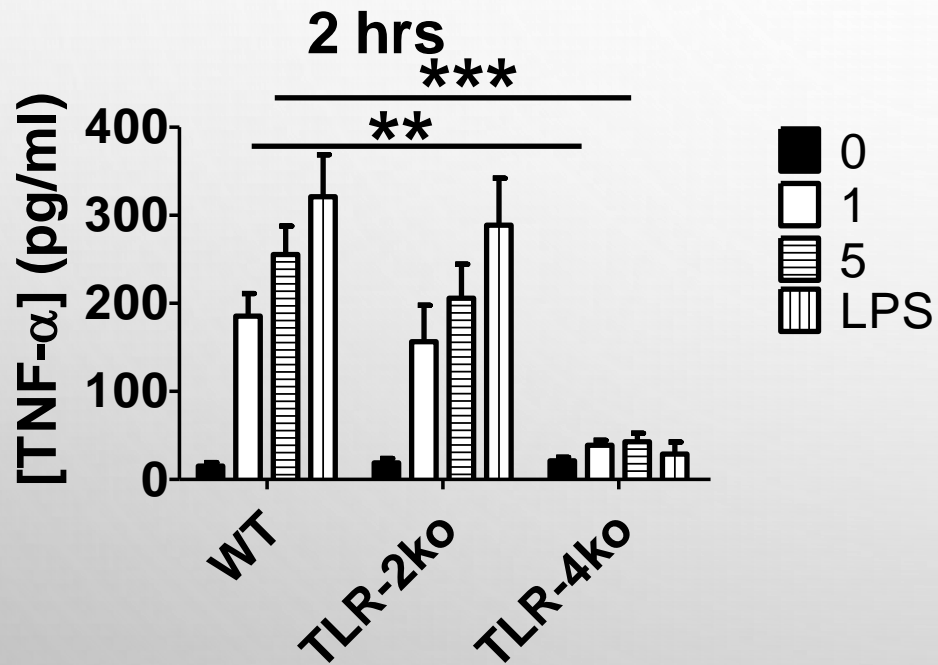
# BIOLOGICAL EVALUATION

## Innate Immunity: TLR2 vs TLR4 Ligand

TO ELUCIDATE THE BACTERIAL TOLL LIKE RECEPTOR RESPONSIBLE FOR CYTOKINE PRODUCTION, THE PERITONEAL MACROPHAGES FROM TLR2 AND TLR4 KNOCKOUT MICE (FEMALE, 4-6 WEEK OLD C57BL6) WERE DOSED WITH COMPOUND 2 AND, TNF-A AND IL6 LEVELS WERE MEASURED OVERTIME IN THE SUPERNATENTS.

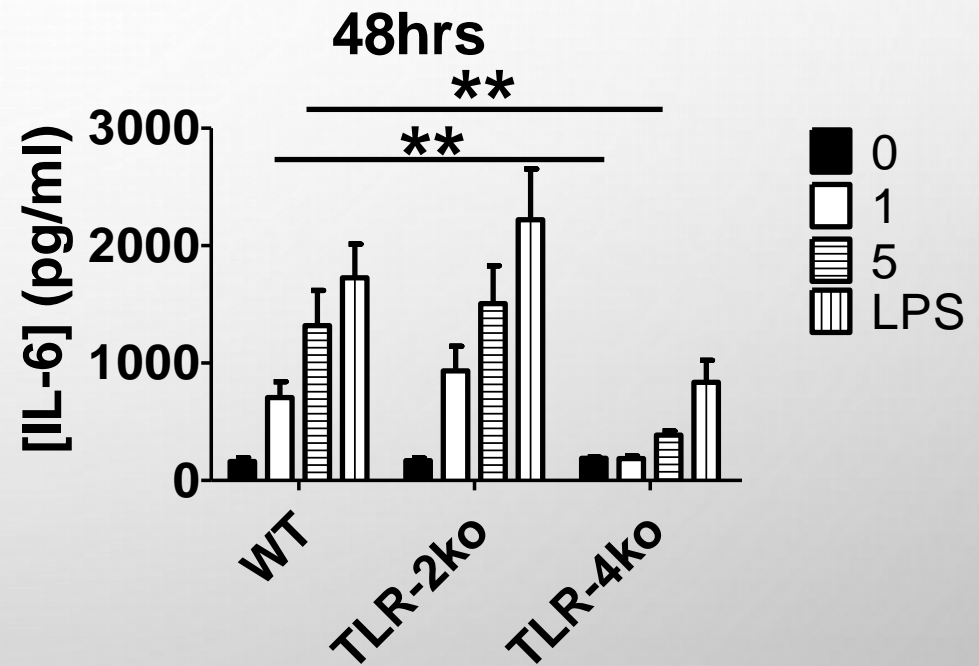
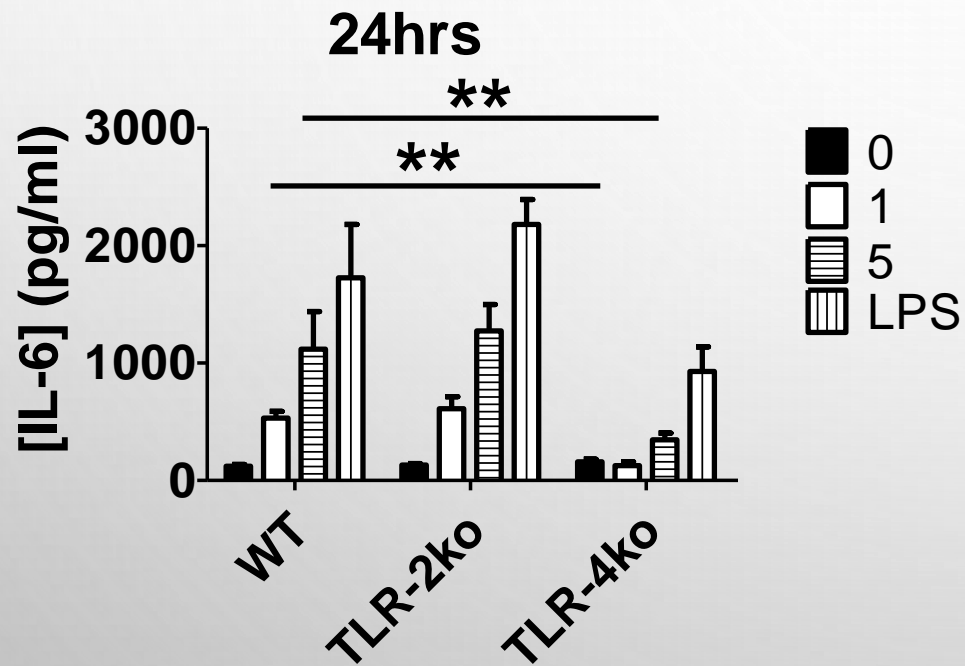
# BIOLOGICAL EVALUATION

## TLR KO MICE: TNF- $\alpha$ Production



# BIOLOGICAL EVALUATION

## TLR KO MICE: IL-6 Production



# BIOLOGICAL EVALUATION

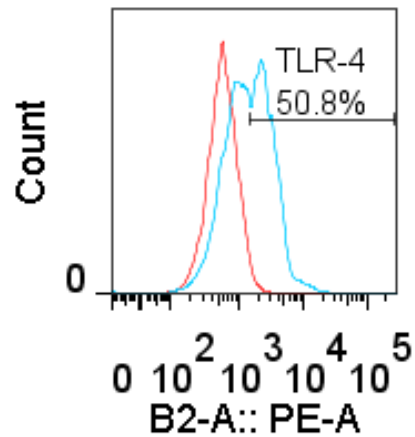
## TLR-4 EXPRESSION: Flow Cytometry

THE EXTENT OF BINDING OF ANALOGUE 2 TO THE TLR-4 RECEPTORS WAS ILLUSTRATED BY TREATING THE PERITONEAL EXUDATE CELLS (PEC) WITH DIFFERENT CONCENTRATIONS OF THE COMPOUND. THE CELLS WERE PRE-INCUBATED WITH THE COMPOUND, AFTER WHICH WERE WASHED. TO THE WASHED CELLS WAS ADDED THE ANTIBODY FOLLOWED BY THE PHYTOERYTHRIN DYE AND, THE NUMBER OF THE COLOURED CELLS WAS OBSERVED BY FLOW CYTOMETRY.

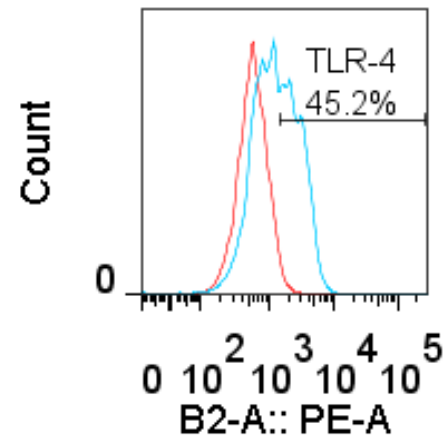
# BIOLOGICAL EVALUATION

## TLR-4 EXPRESSION: Flow Cytometry

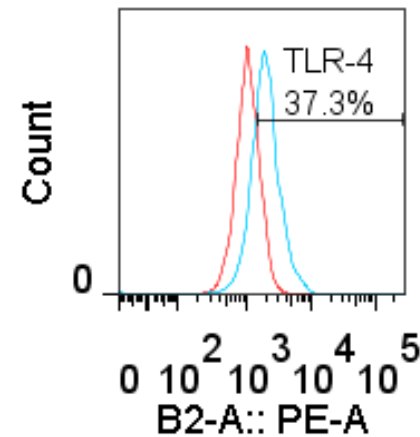
TLR-4 ctrl.



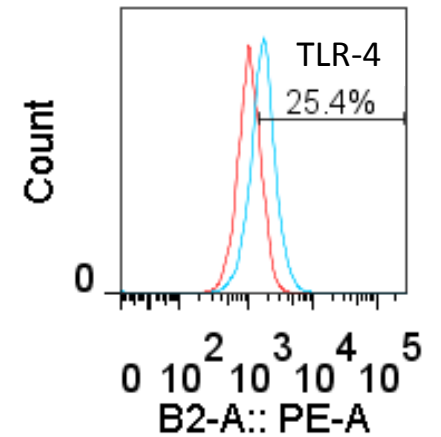
TLR-4 + 1ug block



TLR-4 + 5ug block

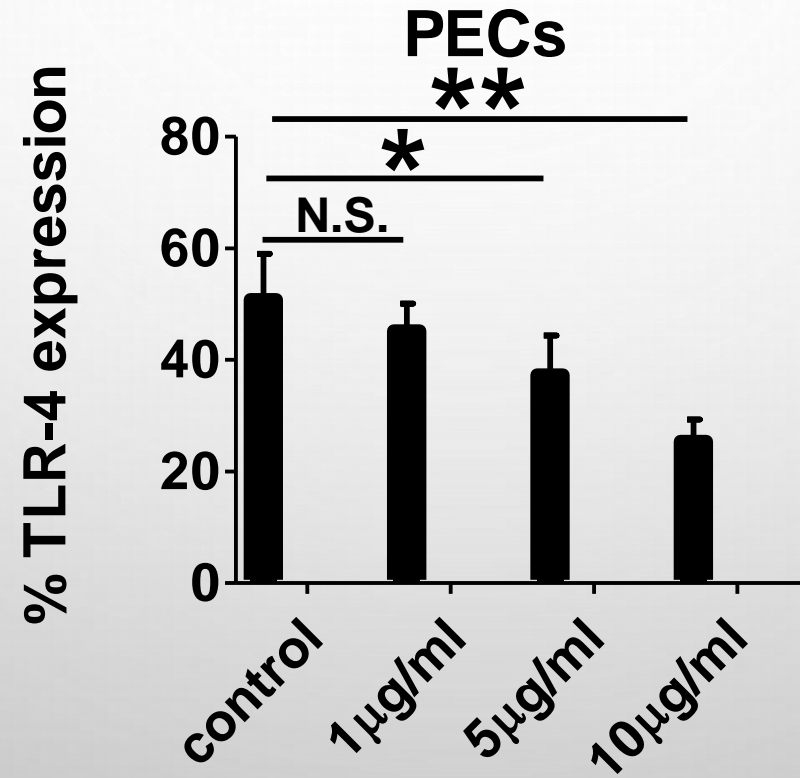


TLR-4 + 10ug block



# BIOLOGICAL EVALUATION

## TLR-4 EXPRESSION: Flow Cytometry

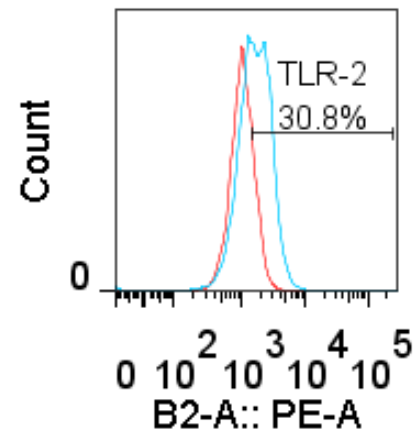




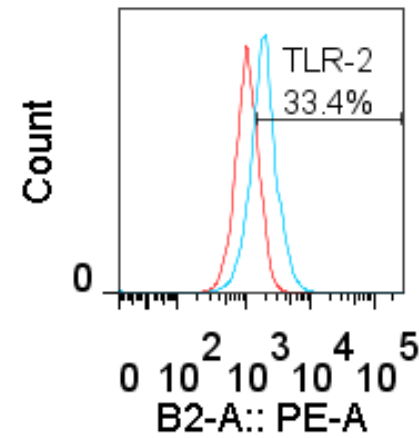
# BIOLOGICAL EVALUATION

## TLR-2 EXPRESSION: Flow Cytometry

TLR-2 ctrl.



TLR-2 + 10ug block



# BIOLOGICAL EVALUATION

## SUMMARY: TLR KO MICE

- A REDUCTION IN THE PRODUCTION OF BOTH TNF- $\alpha$  AND IL-6 WAS OBSERVED IN ONLY THE TLR4 KNOCKOUT MICE SUGGESTING THAT SIGNALING OF  $\alpha$  OCCURS THROUGH TLR4. FURTHERMORE, THE MAGNITUDE OF TLR4 SIGNALING WITH  $\alpha$  WAS GREATER THAN THE KNOWN TLR4 AGONIST, *E. COLI* LPS (LIPOPOLYSACCHARIDE)

# CONCLUSIONS

- THE DESIRED ZWITTERIONIC POLYSACCHARIDE, AN ANALOGUE OF PSA1, WAS SUCCESSFULLY SYNTHESIZED WITH BOTH AMINO AND CARBOXYLATE MOIETIES
- THE BIOLOGICAL EVALUATION CLEARLY DENOTES THAT THE ANALOGUE 2 SHOWS IMMUNOSTIMULATORY PROPERTIES
- ANALOGUE 2 WAS FOUND TO STIMULATE THE INNATE IMMUNE SYSTEM, AND NOT THE ADAPTIVE IMMUNE SYSTEM
- AN UNEXPECTED AND AN EFFICIENT SYNTHESIS OF 3, 6-ANHYDRO-D-GLUCAL PAVES A ROUTE TO THE SYNTHESIS OF INTERESTING NEW CARBOHYDRATE MOIETIES