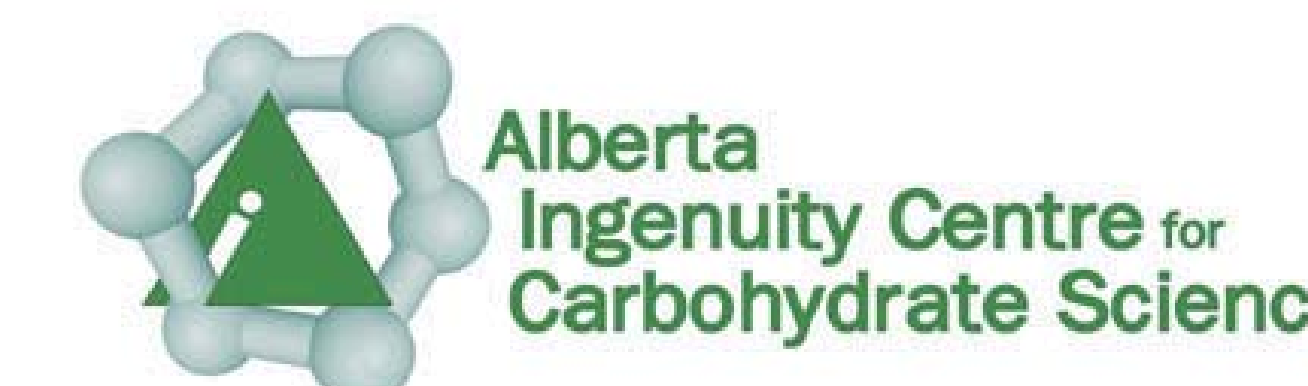




# Chemical Synthesis and Adjuvanticity of Gentiobiosyl Glycerolipids.

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## Introduction

One of the ongoing challenges in the development of conjugate vaccines is the production of an effective adaptive immune response. Immunostimulatory molecules or adjuvants are often employed to initiate the T-cell dependent response.

Recently, Sprott *et al.* have identified that the total polar lipid (TPL) extract from the bacterial strain *Methanobrevibacter smithii* forms liposomes. When compared to conventional liposomes and alum, encapsulated antigen (Ag) induced stronger humoral, cell-mediated and memory responses.<sup>1</sup> This bacterium has evolved to live in high temperature and high acidic environments and contains unique lipid membranes. These long chain isoprenoid ether structures with a glycerol backbone are thought to be crucial for their survival and possibly their immunostimulatory properties.

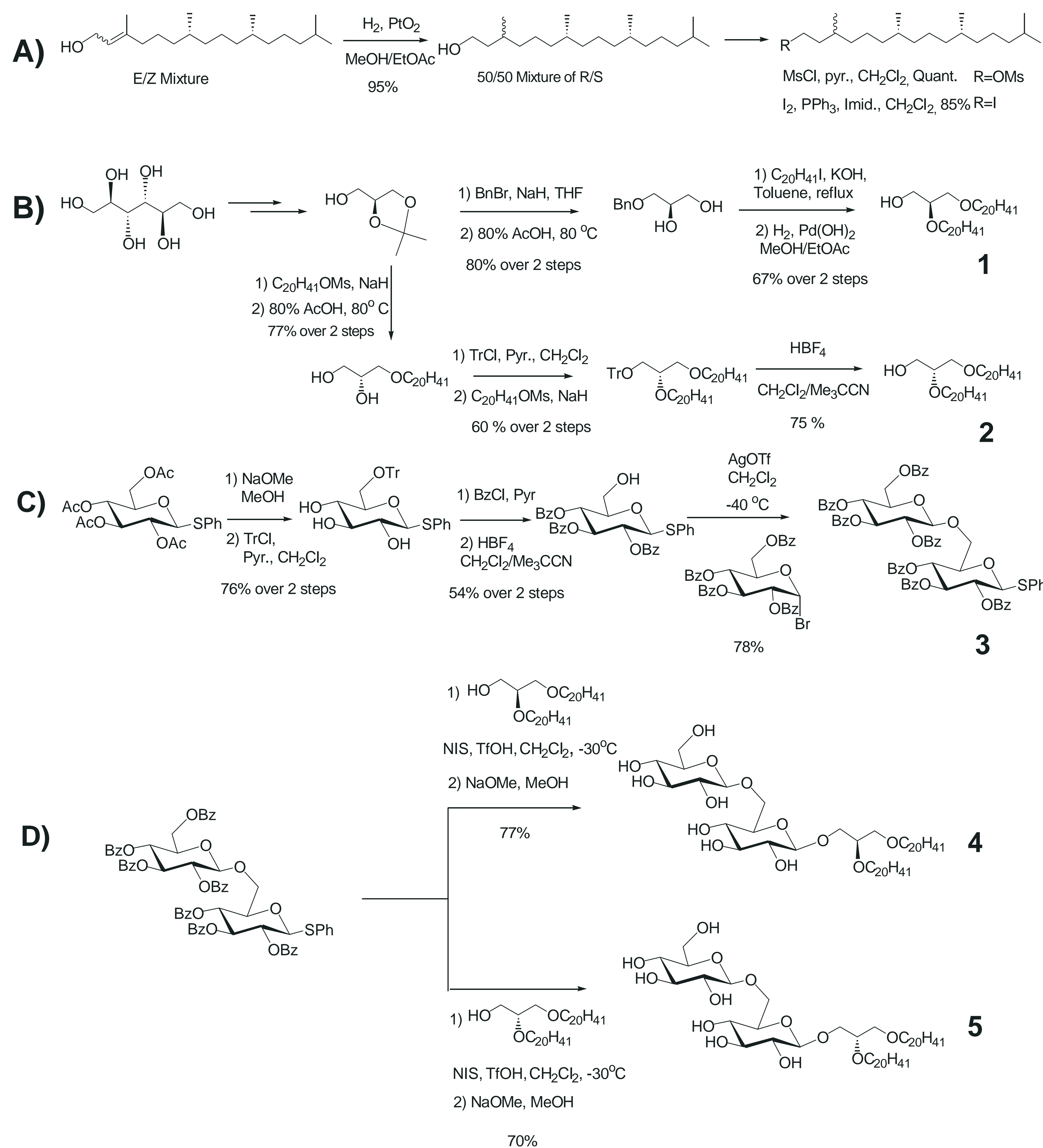
The purpose of this research is to chemically synthesize and individually evaluate the adjuvant properties of the major components of the TPL extract from *Methanobrevibacter smithii*. Herein, we report the synthesis and adjuvant properties of the (2*R*) and (2*S*)-2,3-Bis[(3*R*,5*R*,11*R*)-3,7,11,15-tetramethylhexadecyloxy]propan-1-yl 6-O-β-D-glucopyranosyl-β-D-glucopyranosides **4** and **5**.

## Synthesis and Adjuvant Evaluation

The synthesis of the target glycolipids **4** and **5** were completed using precursors **1**, **2**, and **3** (Scheme 1).

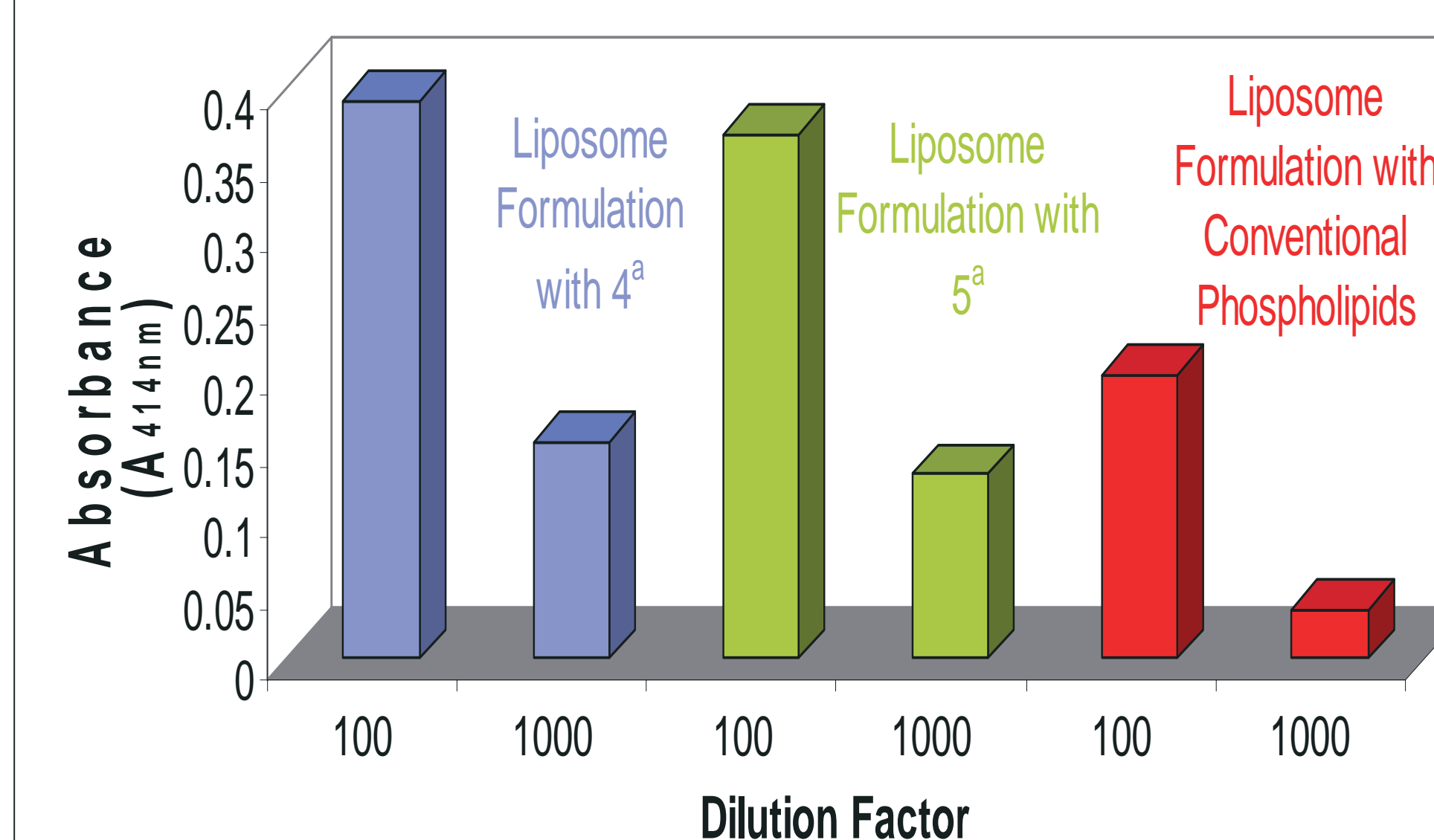
The fully deblocked compounds were used to separately prepare liposomes encapsulating ovalbumin. The antigen loaded liposomes were then injected into mice to elicit an humoral immune response, from which an assessment of the adjuvant properties could be measured via quantification of the amount and subclass distribution of antibody in murine sera.

## Scheme 1: Synthesis of the Target Glycolipids.



**References:** 1. a) G. Dennis Sprott *et al.* *Biochim. et Biophys. Acta*, 1999, 1440, 275-288. b) Lakshmi Krishnan, Chantal J. Dicaire, Girishchandra B. Patel, and G. Dennis Sprott. *Infect. and Immun.*, 2000, 54-63. c) Lakshmi Krishnan, Subash Sad, Girishchandra B. Patel, and G. Dennis Sprott. *J. Immunol.*, 2000, 165, 5177-5185.

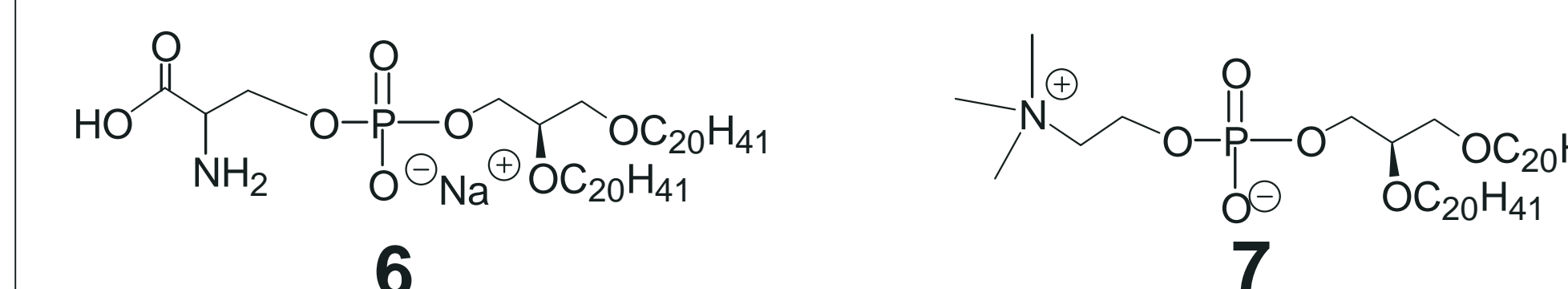
## Humoral Response to Encapsulated Ovalbumin



**Figure 1:** BALB/c mice were immunized with liposomes that encapsulated ovalbumin. Mice were injected on day 0 and day 21 and antigen dose ranged from 8 to 25 ug/dose. Anti-ovalbumin antibody was measured by ELISA on day 31.

a. The lipid/phospholipid mixture for liposome formulation of **4** and **5** consisted of 20 mol% of **4** or **5**, 30 mol% of PC, 20 mol% of DSPC, and 30 mol% of Cholesterol.

## Figure 2: Current Targets.



## Conclusion

-As indicated by the ELISA results, the immunostimulatory capability of **4** and **5** is minimal (Figure 1) and shows no IgM to IgG class switch.

-The investigation will now focus on the synthesis of other components of the TPL extract as well as producing a liposome free of traditional phospholipids.

-In particular, the synthesis of a phosphoserine glycerolipid **6** and the archaeal equivalent of phosphatidyl choline **7** (Figure 2).

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