Synthesis of candidate Natural Killer T cell ligands utilizing novel tandem Staudinger and aziridine formation reaction

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Project Purpose:

To synthesize and characterize new potential Natural Killer T cell ligands to help pinpoint the identity of the endogenous ligand and to elucidate structure-activity relationships between the ligand and its receptor.

Project Importance:

Glycolipids interact with natural killer T cells (NKT cells) via the CD1D receptor, a major histocompatibility complex-like molecule, which triggers responses from NKT cells as the bridge between the adaptive and the innate immunities. Glycolipids have been thought to exist endogenously only in beta configurations in mammals, yet recent studies indicate that alpha glycolipids exist in mammals and interact with NKT cells, which suggests the presence of unknown glycolipid biosynthesis pathways. Whether these alpha glycolipids are synthesized by an unfaithful enzyme or undergo a novel biosynthetic pathway has not yet been determined. We previously observed that some glycolipid species undergo a cyclization reaction in acidic environments, and it is possible that this reaction occurs in the acidic lysosome. We are synthesizing twelve new variants of glycolipid that will be used to determine how cyclization impacts NKT cell stimulation and if cyclized glycolipids are part of the repertoire of endogenous glycolipids.

Project Overview:

Due to purification limitations in isolating natural biochemical products, we will utilize synthetic chemistry to produce twelve new glycolipid species. Some variants of these molecules have already been shown to stimulate NKT cells (Kain, 2014), but further testing is required to determine the range of endogenously produced glycolipids. In the lab we have observed that some of these glycolipids will curiously undergo cyclization into three and five member rings in the presence of an acid. It is possible that the biosynthetic pathway passes through the lysosome, which degrades endocytosed foreign material in antigen presenting cells (Brennan, 2011). Therefore, we hypothesize that the endogenous ligand could be a cyclized variant. To test our hypothesis, I will synthesize twelve cyclized glycolipid variants divided into 3 structural groups.

Group 1: (4 molecules) Beta-linked glycolipids with either glucose or galactose. Three and five-member ring configurations will be synthesized as well, which will produce four total variants. The synthetic pathway for the glucose-linked, three-membered variant is shown in figure 1. The pathway is similar for the other three with minor differences in yields. The reagents used in the steps are as follows:

a) K_2CO_3, trichloroacetonitrile, DCM
b) TMFOTf, DCM, 4AMS
c) NaOMe, MeOH, THF.
d) Ph_3P, Pyridine, 80^0C
e) Nervonic Acid, EDCI, HOBT, THF
f) NaOMe, MeOH, THF

Group 2: (4 molecules) Alpha-linked glycolipids with the same variations as those for beta linked. The pathway for the glucose-linked, three-membered species is shown in Figure 2. The reagents are as follows:

a) Ph_2SO, TTBP, Tf_2O
b) NaOMe, MeOH, THF
c) Ph_3P, Pyridine, 80^0C
d) Nervonic Acid, EDC, HOBT, THF
e) CAN, H_2O, Acetonitrile

Group 3: (4 molecules) single lipid chain variants with either alpha or beta configurations and glucose or galactose-linked (synthesis not shown). These variants are isolated after steps (d) and (c) in the beta and alpha synthesis procedures respectively. The benzoyl and para-methoxybenzyl protecting groups are removed to produce the final compounds.

Figure 1

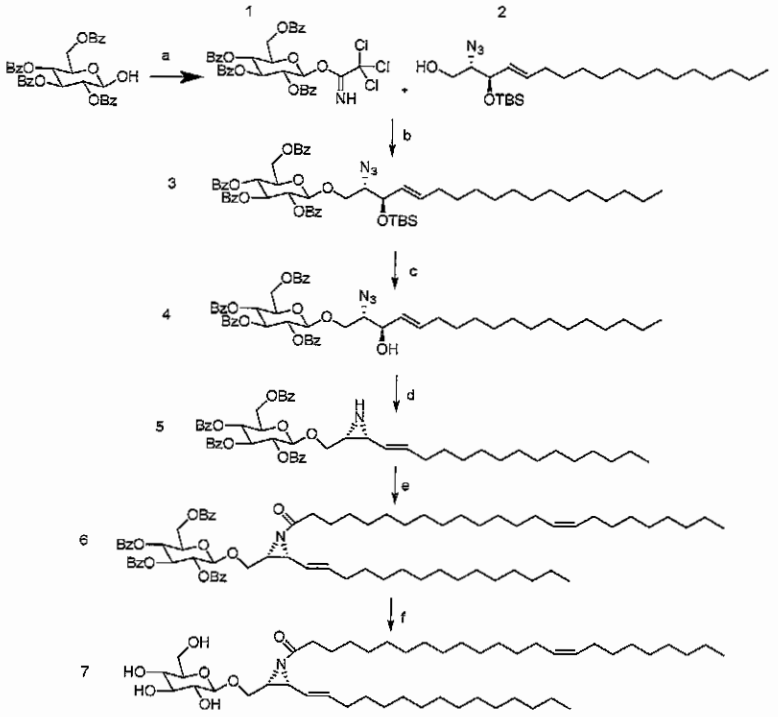
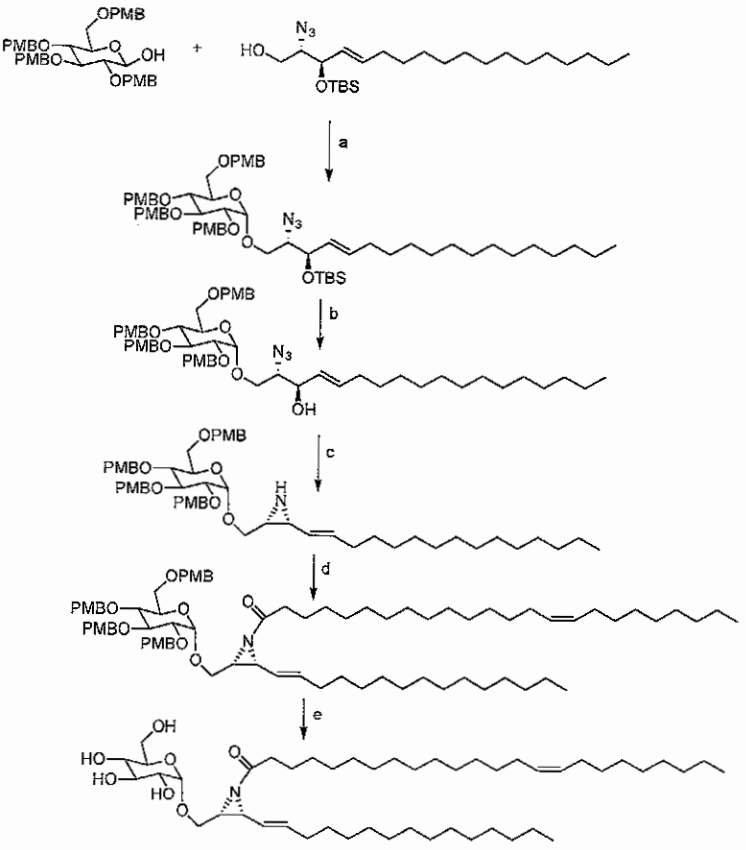


Figure 2



Once all twelve of the NKT cell ligand variants have been synthesized, we will send the compounds to our collaborators to be tested in rodents. By measuring the levels of cytokines—molecules released from immune cells upon stimulation—that are specific to NKT cells we can determine if our compounds are effective.

Qualifications of Thesis Committee:

Dr. Paul Savage – Faculty advisor

I took two chemistry courses from him and am currently enrolled in one more. I have also been working in his lab for a year.

Dr. Jonathon Hill – Reader

I took his cell biology class and mentored in his laboratory for three months.

Dr. Merritt Andrus – Department Honors Coordinator

Timeline:

August 2016 – Begin synthesis of intermediate compounds

July 2017 – begin coupling reactions

January 2018 – Finish synthesis and send compounds to collaborators

February 2018 – complete write up of the chemical aspect of thesis (to submit to Honors program)

- April 2018 – complete write up once collaborators return data and submit research for publication

Culminating Experience:

I have already presented my abstract and preliminary results at the Student Research Conference held at BYU this year (2017). I am also scheduled to present my research at the American Chemical Society's Rocky Mountain Regional Meeting in Loveland, Colorado on October 26th.

References

Brennan, Patrick J. "Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals." *Nature Immunology*, vol. 12, no. 12, Dec. 2011.

Brennan, Patrick J. "Activation of iNKT cells by a distinct constituent of the endogenous glucosylceramide fraction." *Proceedings of the National Academy of Sciences*, vol. 111, no. 37, Feb. 2014, pp. 13433–13438., doi:10.1073/pnas.1415357111.

Kain, Lisa. "The identification of the endogenous ligands of natural killer T cells reveals the presence of mammalian α-Linked glycosylceramides." *Nature Immunity*, vol. 41, 16 Oct. 2014, pp. 543–554.