# Synthetic Studies Toward Lewis Y Glycoconjugates

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## Introduction

Lewis Y (Le$^y$) is a tumour-associated, tetrasaccharide antigen expressed on the surface of various tumour cells. The antigen is a target of therapeutic monoclonal antibodies (BR96) and is proposed as a glycoconjugate cancer vaccine.[1] Active research on synthetic cancer vaccines has yet to establish the optimal structure for the presentation of synthetic carbohydrate antigens to the immune system.

As a step towards resolving the effect of antigen size on vaccine immunogenicity, two extended Le$^y$ epitopes have been synthesized and will be conjugated to BSA (**1** and **2**) .[2-4] By incorporating portions of lactose and a truncated ceramide, the extended antigen approximates the Le$^y$ epitope as it occurs in glycolipids (Fig. 1) on the surface of tumor cells (Fig. 2).

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2. Fernandez-Santana, V., Gonazalez-Lio, R., Sarracent-Perez, J., Verez-Bencomo, V. *Glycoconjugate J.* **15**, 549-553 (1998)
3. Jue, R., Lambert, J. M., Pierce, L. R., Traut, R. R. *Biochemistry* **17**, 5399-5406 (1978)
4. Rose, B. G, Kamps-Holtzapple, C., Stanker, L. H. *Bioconjugate Chem*. **6**, 529-535 (1995)

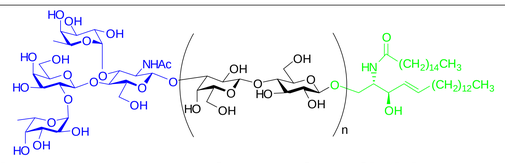


**Figure 1. Natural Le$^y$ Glycolipid**

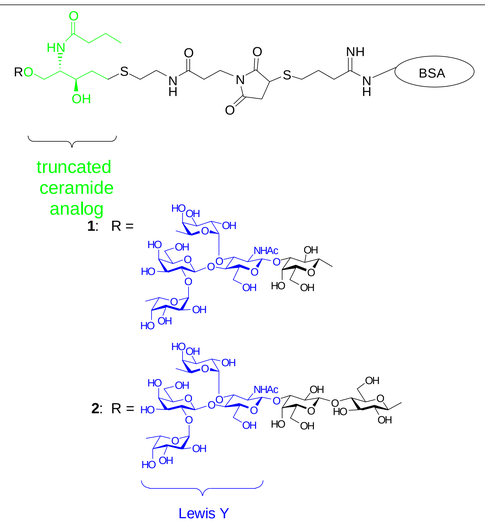


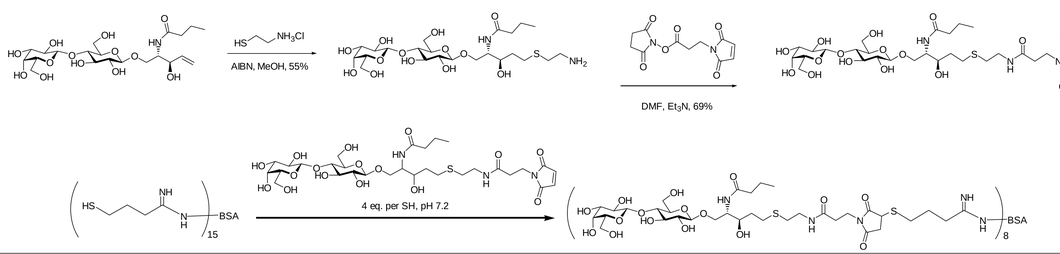

**Figure 2. Target Glycoconjugates**

## Discussion

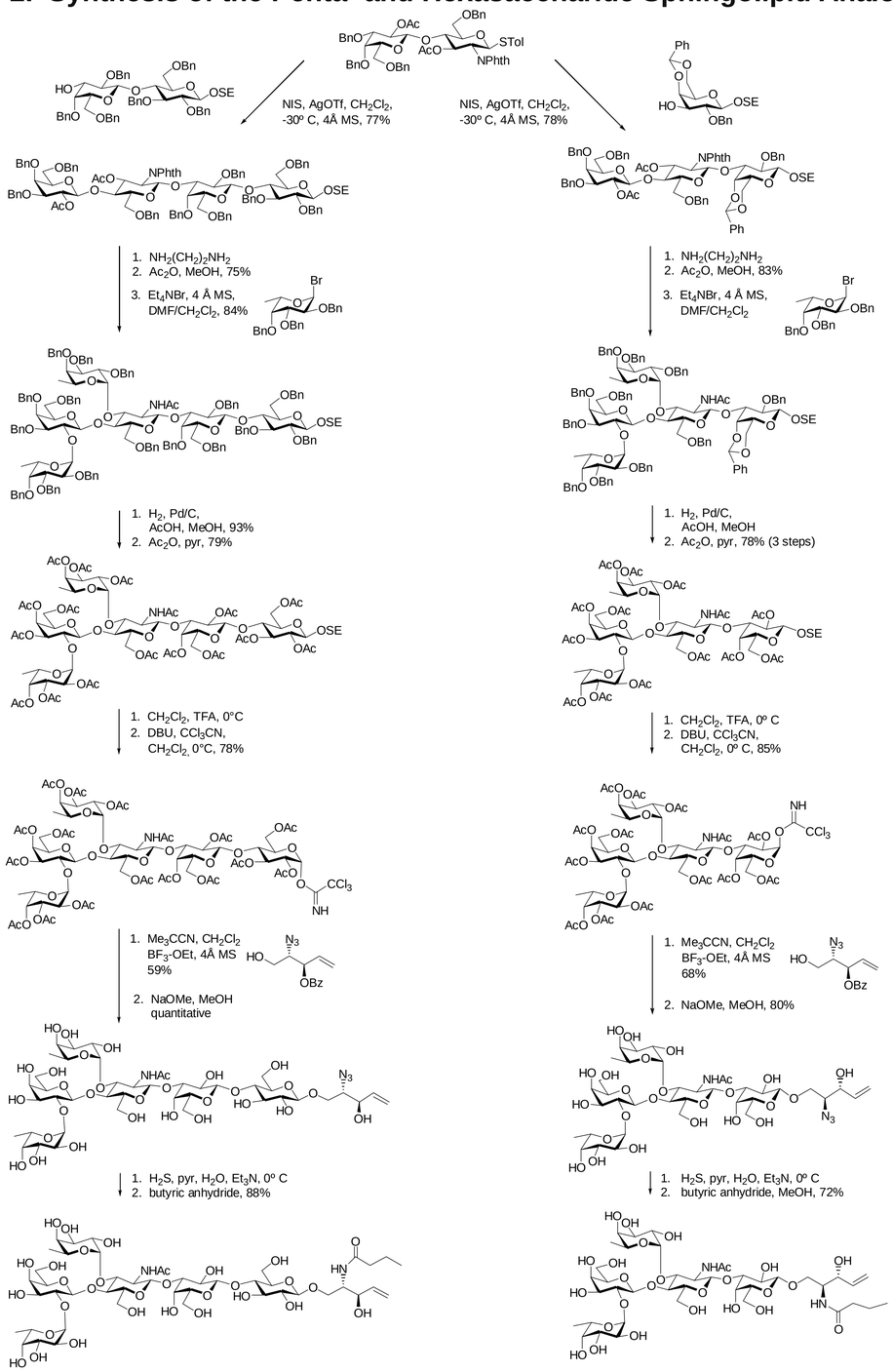
The two Le$^y$ oligosaccharides were synthesized first as TMS ethyl glycosides (Scheme 2). Conversion of the TMS ethyl glycosides to trichloroacetimidate donors was followed by glycosidation with a truncated azidosphingosine acceptor. Reduction of the azide followed by *N*-acylation furnished the desired ceramide analog. This procedure not only provides a ceramide functionalized for coupling to protein but also permits the selection of *N*-acyl groups of different chain length. Since glycosylation of ceramide derivatives is a notoriously low-yielding reaction, truncated azido sphingosines conserve valuable oligosaccharide intermediates.

Protein conjugation experiments have been carried out on a lactose ceramide (Scheme 1). An amino functionality was introduced into the glycolipid analog by radical addition of cysteamine hydrochloride to the alkene. The amino-derivatized sugar was then coupled to the thiolated protein carrier (BSA) using a heterobifunctional cross-linker. The synthetic Le$^y$ glycoconjugates **1** and **2** will be evaluated in mice.

## Scheme 1. Protein Conjugation Studies With A Disaccharide Sphingolipid Analog




## Scheme 2. Synthesis of the Penta- and Hexasaccharide Sphingolipid Analogs

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