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A Strategy for Ready Preparation of Glycolipids for Multivalent Presentation

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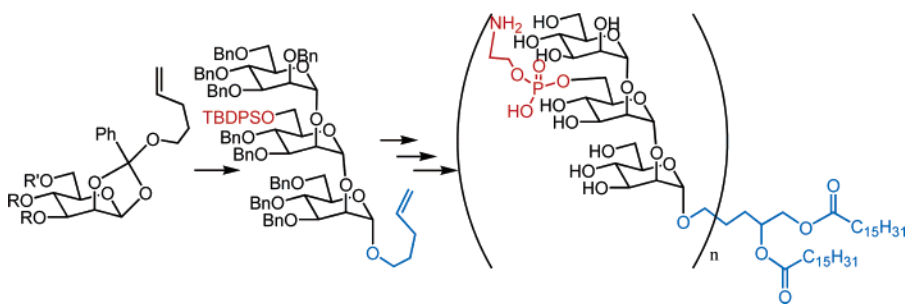
ABSTRACT



The olefinic residue of *n*-pentenyl glycosides serves as the trigger for regioselective construction of higher saccharides and then for elaboration in multivalent glycolipids.

In recent years glycolipid presentation by CD1 protein has emerged as an important aspect of antigen recognition.[1,2] Thus, NKT cells are characterized by a narrow T cell antigen receptor (TCR) repertoire that recognizes glycolipid antigens, resulting in the formation of monomorphic CD1d antigen-presenting cells, which have a unique capacity to rapidly produce large amounts of both T helper (Th) 1 and Th2 cytokines. Major interest therefore centers around the roles that CD1 molecules play in host defense[3] by controlling the development and function of T cell receptors. Their mode of action is consistent with a structural architecture that possesses hydrophilic and hydrophobic domains.[4−6] The lipids that were studied originally were mycolic acids. Moody et al.[7] found that a variety of lipid antigens were bound "relatively nonspecifically", there being no apparent dependence on the structural variations in the hydrophobic tails of the test candidates. Indeed a timely crystal structure of a member of the CD1 family by Zeng et al.[8] revealed the

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presence of a deep hydrophobic groove that could accommodate the aliphatic moiety of a glycolipid.

The glycolipids that have provided the experimental basis for interpreting CD1 function include glycosylphosphatidyl inositols (GPIs),[9,10] lipoarabinomannans (LAMS),[5] and other mycobacterial specimens. These phosphoinositides are associated with some of the world's oldest, most dreaded, and most obdurate diseases, including malaria and tuberculosis. The synergy of the latter with HIV infection[11] raises the dread level manyfold.

These biological insights come amidst structural insights into the importance of multivalent presentation of carbohydrate epitopes. Thus, Lee's seminal experiments[12] on the biological effect of sugar clusters laid the foundation[13] for the concept of multivalency and hence for a rational approach to the synthesis of glycolipid test candidates that are amenable to supramolecular assembly.

Our long-standing synthetic work on phosphoinositides,[14] GPIs[15] and mycobacterial glycolipids[16] has sensitized us to CD1 science, and fortunately, our synthetic strategies lend themselves to the preparation of candidates for multivalent presentation. In this manuscript we report some ready routes.

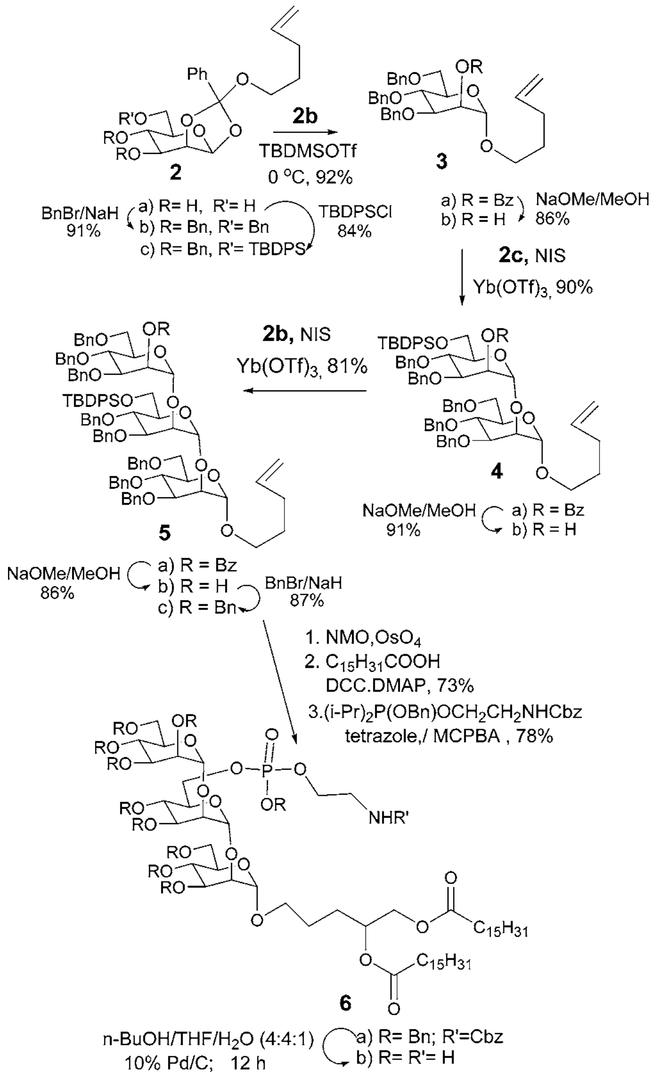
At the distal end of membrane-anchored GPIs (e.g., **1**)

protein-EtN-P-6(Manα1-2)Manα1-2Manα1-6Manα1-4GlcN-PI
1

is a trimannan array, the middle unit of which bears a phosphoethanolamine residue.[17] A protein is sometimes attached to the latter; however, Ralton and McConville have noted that "a significant proportion of GPI synthetic output of a cell is NOT directed to protein anchoring," which raises questions as to their purpose.[18]

Our synthesis of the distal trimannan construct **7** described in Scheme 2 employed the readily prepared *n*-pentenyl ortho

Scheme 1



ester (NPOE) **2a** as a convenient sole source progenitor. Perbenzylation gave **2b**, while selective silylation prior to benzylation gave **2c** (Scheme 1). Treatment of **2b** with TBDMSOTf brought about rearrangement of the ortho ester[19] and provided **3b** in near quantitative yield. The *n*-pentenyl glycoside (NPG) **3b** and NPOE **2c** are both potential glycosyl donors, but because $Yb(OTf)_3$/NIS reacts chemospecifically with NPOEs,[19] formation of disaccharide **4a** was assured. Removal of the benzoate group afforded **4b**, and a second chemospecific reaction, this time with **2b**, gave the desired trisaccharide **5a**, from which the benzylated counterpart **5c** was obtained by routine transformations.

The chemoselectivity in the reaction of precursors **2c** and **3b** was now appreciated since the olefinic residue of **5c** could be dihydroxylated, esterified, and desilylated, which allowed insallation of the phosphoethanolamine residue in **6a**. Hydrogenolysis then afforded compound **6b**.

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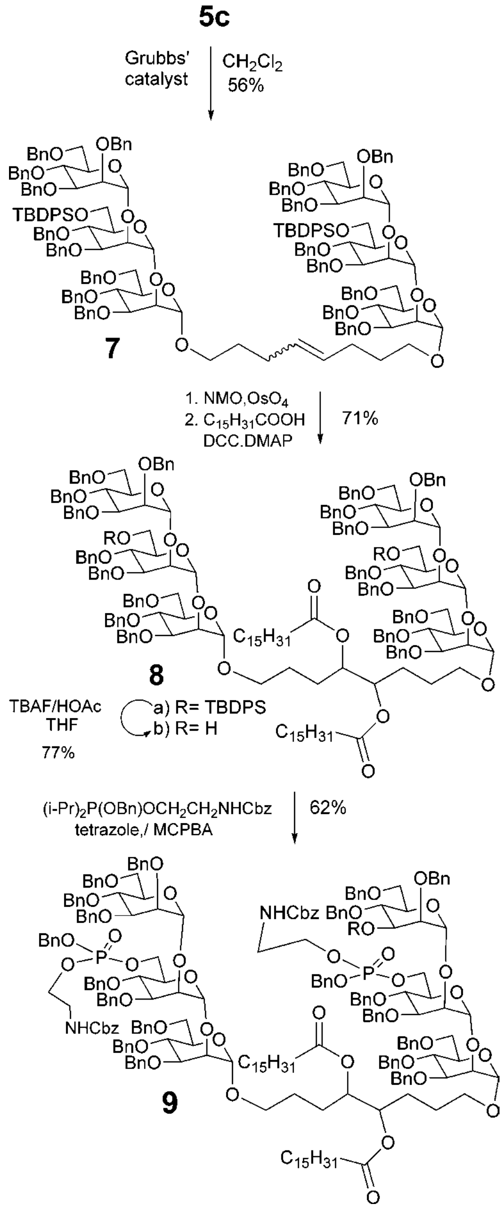
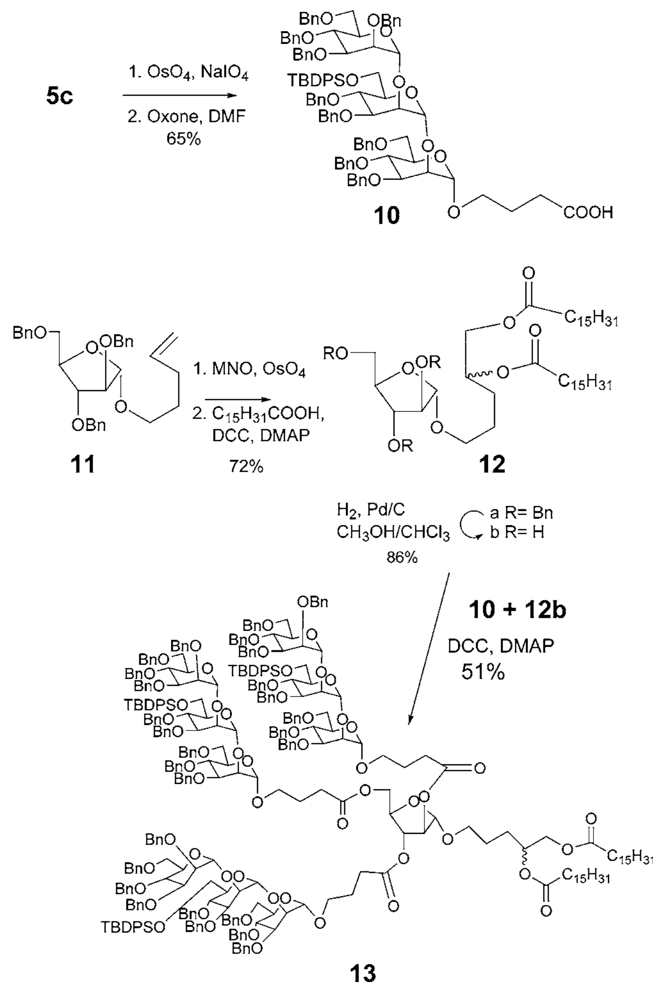
Scheme 2



Scheme 3



The *n*-pentenylated trisaccharides **5c** also served as precursor for the di- and trivalent neoglycolipids. Thus, Grubb olefin metathesis[20] provided the bis-disaccharide **7**, which was subjected to similar steps as described above, to obtain the divalent construct **8**. The latter was then processed, along the lines in Scheme 2, to give the bis-phosphodiester **9**.

Our strategy for the trivalent construct made use of two *n*-pentenyl glycosides in different ways. First, the olefinic unit of **5c** was cleaved to provide the carboxylic acid **10**. Monosaccharides have served as scaffolds for displaying bioprobes of various sorts,[21] and so we made use of our recently synthesized[22] *n*-pentenyl arabinoside **11** (Scheme 3). Hydroxylation and esterification of the resulting diol gave the diester **12a**, and hydrogenolysis freed the hydroxyl groups in **12b**. The latter was then acylated with the glycoconjugate **10** under the agency of DCC and DMAP to produce compound **13**.

The strategy for the di- and trivalent presentations in Schemes 2 and 3 are different and illustrate the versatility of *n*-pentenyl derivatives for ready preparation of multivalent constructs. Our studies along this line are continuing, and the biological effects of these multivalent constructs are currently being tested.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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