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Two-Step Synthesis of the Immunogenic Bacterial Glycolipid BbGL1

Suvarn S. Kulkarni and Jacquelyn Gervay-Hague*

University of California, Davis, Department of Chemistry, One Shields Avenue, Davis, California 95616

gervay@chem.ucdavis.edu

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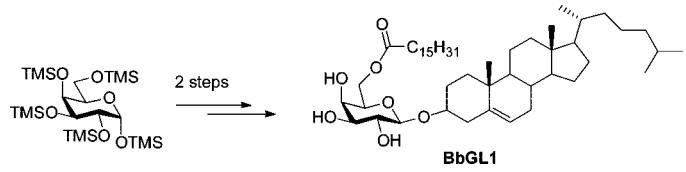
ABSTRACT



Chemical synthesis of a bacterial glycolipid BbGL1 is reported in two steps starting from per-*O*-TMS D-galactose. The key features are glycosyl iodide mediated β-stereoselective glycosylation in the absence of neighboring group participation and regioselective acylation.

In 2001, two major glycolipids were isolated from *Borrelia burgdorferi*, the causative agent of Lyme disease, which is a multisystemic disorder affecting the skin, nervous system, heart, and joints.[1] These glycolipids were initially characterized as galactosyl diacyl glycerol. Two years later, the structures of these highly immunoreactive glycolipids were corrected as cholesteryl 6-*O*-acyl-β-D-galactopyranoside (BbGL1) **1** and 1,2-di-*O*-acyl-3-*O*-α-D-galactopyranosyl-*sn*-glycerol (BbGL2) **2** (Figure 1). The major fatty acids were palmitate and oleate.[2] Little is known about the protective antigens or the host factors of *B. burgdorferi*. Glycolipids **1** and **2**, being the only antigenic lipid components of *B. burgdorferi*, are looked upon as valuable candidates for diagnosis and potential vaccines against Lyme disease.[3] Moreover, BbGL1 is thought to be involved in developing host immunity during Lyme disease.[4] Chemical synthesis offers an opportunity to obtain large quantities of these structurally well-defined glycolipids in high purity for immunological studies.

In the past few years, we have demonstrated the utility of glycosyl iodides in the synthesis of α-*O*-glycosides.[5] Recently, we developed a highly efficient one-pot protocol for the synthesis of α-linked glycolipids including BbGL2 and its analogs.[6] A short and general strategy to access *C*-analogs of BbGL2 was also established.[7] A chemoenzymatic synthesis of BbGL1 was published recently; and the first chemical synthesis of BbGL1 was achieved by Pozsgay and co-workers.[8,9] Their approach relied upon neighboring group participation of a C-2 pivaloyl ester to install the β-cholesteryl linkage and required a number of selective protections to arrive at the final target. In continuation with our work on *Borrelia* glycolipids, herein, we wish to report a concise chemical synthesis of BbGL1 using glycosyl iodides.

Retrosynthetically, the synthesis of the target molecule requires a β-selective glycosidation and regioselective acylation of the C-6 hydroxyl. As noted above, β-selective glycosidation is achieved using neighboring group participa-

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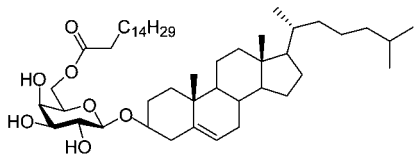
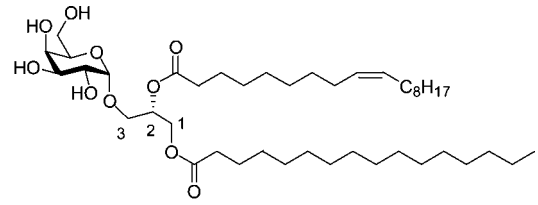


Cholesteryl 6-*O*-acyl-β-D-galactopyranoside
Borrelia burgdorferi glycolipid **1**
(BbGL1)



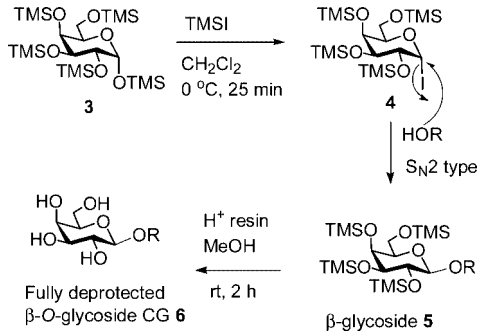
1,2-di-*O*-acyl-3-*O*-α-D-galactopyranosyl-*sn*-glycerol
Borrelia burgdorferi glycolipid **2**
(BbGL2)

Figure 1. Structures of highly immunogenic glycolipids from *B. burgdorferi*.

tion of an ester type group at C2[10] or by using participating solvent such as acetonitrile.[11] However, orthoester formation in the former and imidate formation in the latter are often side reactions. Also the nitrile effect is sensitive to steric constraints in the donor and acceptor pair.[12] Very recently, a *β*-selective glucosidation procedure was reported for thioglucosyl donors bearing 2,3,4-tri-*O*-TIPS groups and a 6-*O*-Piv group employing a conformational switching strategy.[13] Bols and co-workers presented a comprehensive and intriguing study on the conformational change of "super-armed donors" equipped with TBS and TIPS groups.[14] Unfortunately, these conditions are not suitable for galactosyl counterparts, which generate only α-isomers under the conditions. Thus, a *β*-selective galactosidation protocol is highly warranted.

We hypothesized that owing to the highly reactive nature of per-*O*-TMS galactosyl iodide **4**, *β*-selectivity could be achieved via direct S_N2 type displacement of an anomeric α-iodide in the presence of a suitable activator (Scheme 1).

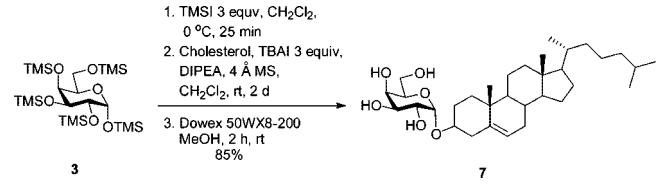
Scheme 1. Hypothesis of *β*-Stereoselective Glycosidation



Upon glycosidation, the TMS groups could then be removed in the same pot under mild conditions to obtain the fully deprotected cholesteryl glycoside (CG) **6**, which could then be acylated at the primary position, regioselectively. Per-*O*-TMS galactoside **3** in turn could be generated quantitatively from D-galactose and used as such without any purification.[15]

To test our hypothesis, we first tried α-stereoselective glycosidation reactions (Scheme 2) using per-*O*-TMS

Scheme 2. α-Glycosidation of Galactosyl Iodide with Cholesterol



galactose **3** and cholesterol under in situ anomerization conditions.[16] We expected α-glycosidation to be highly efficient and felt it would be helpful to have the α-anomer for analysis of *β*-glycosidation reactions.[5−7] Accordingly, compound **3** (3 equiv) was treated with a stoichiometric amount of TMSI at 0 °C in CH_2Cl_2. Upon complete disappearance of the starting material, as indicated by TLC (25 min), the formed glycosyl iodide solution was directly cannulated into a solution of cholesterol (1 equiv), 4 Å molecular sieves, DIPEA, and TBAI (3 equiv) and stirred for two days at ambient temperature. The solvent was evaporated. TBAI was precipitated and filtered using hexane/ethyl acetate, and the crude product was treated with Dowex 50WX8-200 in MeOH for 2 h. Column chromatography afforded exclusively the α-cholesteryl glycoside in 85% yield along with recovery of starting material to the extent of 15%.

We next turned our attention toward achieving *β*-selectivity required for the natural product. Paulsen and others have reported *β*-selective glycosidation in ap-

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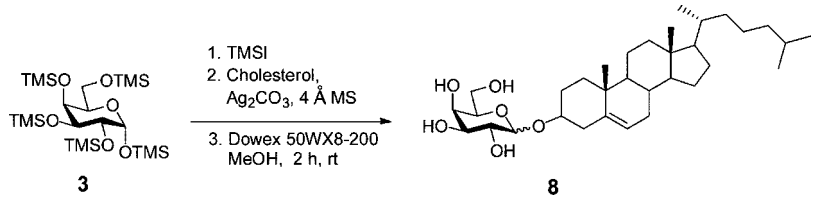
Table 1. β-Glycosidations of Galactosyl Iodide with Cholesterol



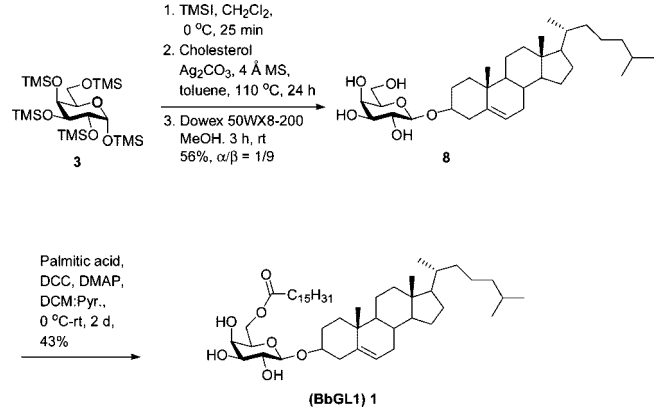
3 8

entry	**3** (equiv)	cholesterol (equiv)	temp (°C)	solvent	time (h)	yield (%)	α/β ratio	recovered cholesterol (%)
1	3	1	rt	CH_2Cl_2	96	50	1/1	49
2	3	1	110	toluene	14	55	1/6	40
3	1	1	110	toluene	3	38	1/6	62
4	3	1	110	toluene	4	46	1/5.5	49
5	1	3	110	toluene	3	43	1/4.5	50
6	3	1	rt	CH_3CN/CH_2Cl_2	50	35	1.6/1	55
7	3	1	110	toluene slower addition	24	56	1/9	42

propriately matched systems using heterogeneous catalysis such as Ag_2CO_3,[17] although what constitutes a matched system is not well understood. We investigated heterogeneous glycosidation of the silyl iodide generated from **3** with cholesterol, as shown in Table 1. Compound **3** (3 equiv) was treated with a stoichiometric amount of TMSI at 0 °C in CH_2Cl_2. Upon completion of the reaction, the so formed glycosyl iodide solution was azeoptroped twice with benzene under reduced pressure and argon atmosphere. The crude yellowish oil was dissolved in CH_2Cl_2 and cannulated into a solution of cholesterol (1 equiv), 4 Å molecular sieves, and Ag_2CO_3 (2 equiv per donor) in CH_2Cl_2 for 4 days (entry 1). The solvent was evaporated, and the residue was subjected to hydrolysis using acidic resin in methanol to give the final products. The reaction furnished an α/β mixture (1:1) of cholesteryl glycosides **7**/**8** (49%) as judged by NMR as well as isolation of the two isomers by per-*O*-acetylation and chromatographic separation; 49% cholesterol was also recovered. To speed up the reaction, it was conducted at toluene reflux temperature overnight. This remarkably improved the α/β selectivity in favor of the desired β-isomer **8**[18] (entry 2, α/β = 1/6, 55%). Changing the stoichiometry of donor/acceptor to 1:1 and reversing it to 1:3 had little impact on the outcome of the reaction (entries 3−5). Hoping to achieve complete selectivity, the reaction was conducted in CH_2Cl_2 using acetonitrile as a cosolvent (entry 6). However, to our surprise, the reaction gave a mixture of **7** and **8** favoring unwanted **7** (α/β = 1.6/1, 35%). Finally, we repeated the reaction in toluene at reflux temperature with slow cannulation of the donor (entry 7). This reaction gave the β-isomer as the major product (α/β = 1/9 from ^{1}H NMR) in 56% overall yields. In all the reactions, cholesterol was recovered, which could be chemically separated from the polar product by washing with a hexane and toluene mixture (1/1). The mixture of isomers could be separated by very careful silica gel column chromatographic separation using 5% MeOH in CH_2Cl_2 as eluent or alternatively by carrying out per-*O*-acetylation separation and deacetylation.

With the requisite cholesteryl glycoside **8** in hand, we proceeded further to test regioselective palmitoylation using DCC as a coupling agent (Scheme 3). The best results were

Scheme 3. Synthesis of BbGL1



obtained using a CH_2Cl_2/pyridine solvent combination (1:1). Compound **8** was dissolved in pyridine and a solution of DCC, cat. DMAP, and palmitic acid in CH_2Cl_2 was slowly cannulated at 0 °C and stirred for 2 days at rt. The reaction cleanly afforded BbGL1 **1** in 43% yield along with the recovery of **8** to the extent of 39%. The results are comparable with the reported enzymatic reaction (palmitic acid vinyl ester, THF, Novozyme, 40 °C, 4 days, 38%, SM recovered 55%).[9] The NMR data corroborated well with those reported in the literature.[2]

In conclusion, a chemical synthesis of immunogenic glycolipid BbGL1 was achieved in two steps starting from known

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per-*O*-TMS galactose in 21% overall yield. The β-selectivity in the key glycosylation was achieved without the participation of the neighboring group or solvent. The synthesis of other significant β-linked glycolipids using the above established methodology and biological evaluation of BbGL1 are currently underway.

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Note Added after ASAP Publication. A description of and reference to a prior synthesis of BbGL1 was not included in the version published ASAP September 18, 2008. References 3 and 9 and extensive text changes were added to the revised version published ASAP October 8, 2008.

Supporting Information Available: General experimental details, experimental data, and ^{1}H NMR and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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