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Two-Step Synthesis of the Immunogenic Bacterial Glycolipid BbGL1

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Abstract

Chemical synthesis of a bacterial glycolipid BbGL1 is reported in two steps starting from per-*O*-TMS β -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

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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 ¹H NMR and ¹³C NMR spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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 participation of an ester type group at C2¹⁰ 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 “superarmed 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). 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 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 CH₂Cl₂. 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 appropriately matched systems using heterogeneous catalysis such as Ag₂CO₃,¹⁷ 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 CH₂Cl₂. Upon completion of the reaction, the so formed glycosyl iodide solution was azeotroped twice with benzene under reduced pressure and argon atmosphere. The crude yellowish oil was dissolved in CH₂Cl₂ and cannulated into a solution of cholesterol (1 equiv), 4 Å molecular sieves, and Ag₂CO₃ (2 equiv per donor) in CH₂Cl₂ 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 CH₂Cl₂ 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 ¹H 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 CH₂Cl₂ as eluent or alternatively by carrying out per-*O*-acetylation separation and deacetylation.

With the requisite cholesterol glycoside **8** in hand, we proceeded further to test regioselective palmitoylation using DCC as a coupling agent (Scheme 3). The best results were obtained using a CH₂Cl₂/pyridine solvent combination (1:1). Compound **8** was dissolved in pyridine and a solution of DCC, cat. DMAP, and palmitic acid in CH₂Cl₂ 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 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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

Scheme 1.
Hypothesis of β -Stereoselective Glycosidation

Scheme 2. α -Glycosidation of Galactosyl Iodide with Cholesterol

Scheme 3.
Synthesis of BbGL1

Table 1*β*-Glycosidations of Galactosyl Iodide with Cholesterol

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	$\text{CH}_3\text{CN}/\text{CH}_2\text{Cl}_2$	50	35	1.6/1	55
7	3	1	110	toluene slower addition	24	56	1/9	42