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Facile Synthesis of a Library of Lyme Disease Glycolipid Antigens

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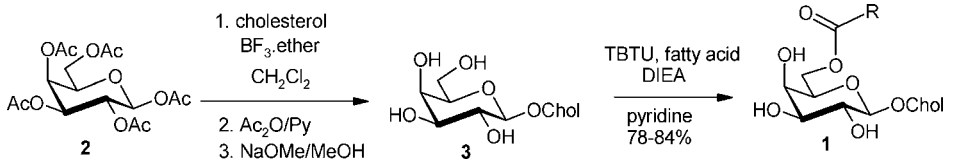
ABSTRACT



A library of one of the two Lyme disease antigens, BbGL1, has been synthesized in four steps from D-galactose using BF_3-promoted glycosylation of the peracetate to introduce the cholesteryl β-glycoside and TBTU-promoted esterification to add a range of fatty acids regioselectively at O-6 of D-galactose in good yield.

Lyme disease (LD) is a rapidly expanding multisystem illness that is the most common tick-bourne disease in the northern hemisphere.[1] It is caused by species of the spirochete *Borrelia burgdorferi senso lato*; at least three species cause the disease in Europe, *B. afzelii*, *B. garninii*, and *B. burgdorferi* senso stricto, but only the latter is important in North America. The bacteria are transmitted to humans by bites of ticks of species of the genus *Ixodes*.[2] These ticks have a three-host life cycle where the larvae feed on small rodents and birds that act as reservoirs for the bacteria.[2a,3] The larvae leave the initial host, then molt into nymphs that feed on a second host, primarily small rodents but also humans, and then leave the second host and molt into adults that feed on large mammals, primarily deer but including dogs and occasionally humans.[2a]

The bacteria reside in the midgut of the ticks and only migrate to the salivary glands after changes in surface protein expression (OspA to OspC) occur induced by a mammalian blood meal. These changes require at least 17 h of feeding.[1b] A vaccine against Lyme disease based on the outer surface protein OspA was introduced in 1998 but withdrawn in 2002 for several reasons among which was the recognition that the change in surface protein expression made it an unusual transmission-blocking vaccine.[4] A vaccine against Lyme disease is considered desirable, but other antigens need to be identified if multiple booster shots are to be avoided.[4] Other possible antigens include two glycolipids first isolated from *B.burgdorferi* in 2001[5] and shown to be cholesteryl 6-*O*-acyl-β-D-galactopyranoside (BbGL1) and 1,2-di-*O*-acyl-3-*O*-α-D-galactopyranosyl-*sn*-glycerol (BbGL2) (see Figure 1),[6] abundant in the outer membranes of the LD-causing *B. burgdorferi* species.[7] The acyl groups in both BbGL1 and BbGL2 are a mixture of palmitoyl and oleoyl groups, and it was recently shown that at least one of the acyl groups in BbGL2 must contain a *cis*-alkene for antigenicity to be maintained.[8]

This publication describes a short efficient synthesis of a library of BbGL1 derivatives that makes use of our

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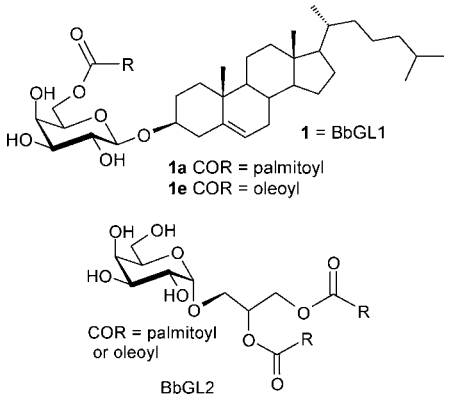


Figure 1. *Borrelia burdorferi* glycolipid antigens.

recently described primary-selective synthesis of esters[9] for the acylation step. BbGL1 and its derivatives have been synthesized five times since its structure was determined[6] in 2003. Pozsgay et al. outlined a 12-step synthesis from D-galactose using pivaloyl groups to achieve β-selective glycosylation via neighboring group participation and protecting group chemistry to introduce the ester group at O-6 in 11% overall yield.[10] This group also reported synthesis of a derivative allowing conjugation to protein using a similar pathway.[11] Wu et al. prepared the palmitoyl version of BbGL1 in low yield (38%) on a small scale enzymically but did not indicate how they prepared the cholesterol glycoside.[12] Kulkarni and Gervay-Hague reported a short synthesis, but the yield of the glycosylation step was moderate (56% β/α 9/1) and that in the DCC-promoted selective esterification step was worse (43%).[13] In addition, Stübs et al. were unable to repeat the carbodiimide-promoted selective esterification reaction, despite extensive studies.[14] This latter group synthesized the cholesteryl glycoside in four steps from galactose and then added a number of different acyl groups enzymically using acyl transfer from acetone oxime esters, catalyzed by a lipase in low yields (5 to 31%).[14]

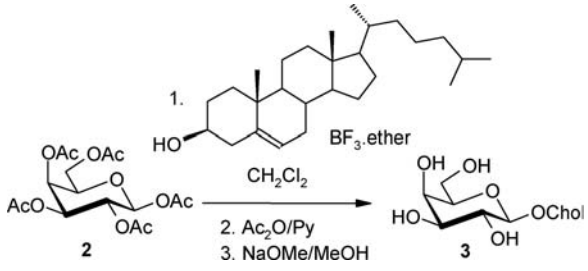
Two key steps are required for the synthesis of BbGL1 analogues from D-galactose: a β-selective glycosylation and regioselective esterification. We opted to perform the glycosylation using the single step Lewis acid catalyzed reaction of cholesterol with easily accessible β-D-galactose pentaacetate (**2**), even though the yield would be expected to be lower than those of the two- or three-step processes involving more active glycosyl leaving groups. Lewis acid catalysis of glycosylation of pentaacetates has been known

Table 1. Effect of Variation of Conditions on Glycosylation

GalPa concn (M)	cholesterol (equiv)	solvent	BF_3 (equiv)	time (h)	yield (%)	β/α
0.087	3.0	DCM	0.5	48	61	5/1
0.032	3.0	DCM	1.5	72	58	6/1
0.032	10	DCM	1.5	72	73	6/1
0.032	10	DCM	3.0	48	62	5/1
0.032	10	DCM	4.5	48	59	3/1
0.01	3	DCM/ MeCN 6:1	1.5	96	51	12/1
0.01	10	DCM/ MeCN 6:1	1.5	96	66	11/1

for some time using acids such as tin tetrachloride[15] and zinc chloride,[16] but we chose to investigate boron trifluoride etherate to avoid the use of toxic or solid hydroscopic metal catalysts. This activator has been used before, but only with primary alcohols for preparative purposes as far as we are aware.[17] Ellervik et al. demonstrated that these reactions proceed rapidly with glycosyl acetates under equimolar conditions to give the desired product accompanied by slower anomerization and equilibration.[18] We investigated the effects of changing conditions as outlined in Table 1. Increasing the relative amount of cholesterol increased the yield. Increasing the amount of BF_3 increased the rate but decreased the β/α ratio. Adding acetonitrile slowed the reaction rate but improved the β/α ratio, although we could not find conditions that eliminated the need for separation of the anomers by column chromatography. The best conditions were in DCM with 1.5 equiv of BF_3 which gave an acceptable 73% yield (β/α 6:1).

Scheme 1. Synthesis of Cholesteryl β-D-Galactopyranoside (**3**)[a]



[a] See Table 1 for reaction times and conditions.

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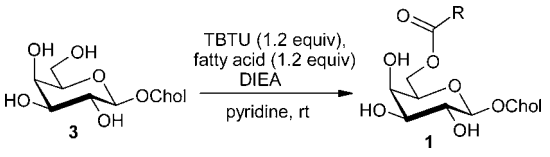
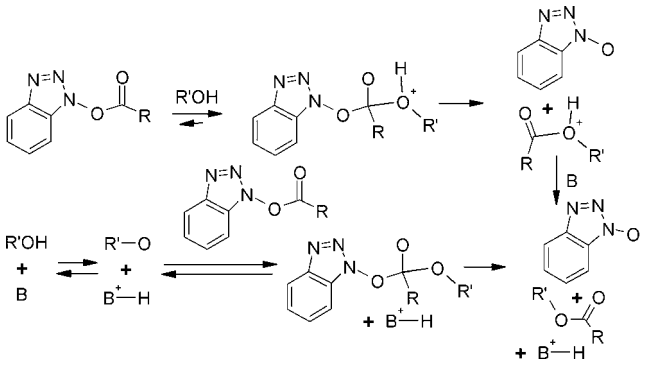
Table 2. Results of Esterification Reactions



fatty acid	DIEA (equiv)	time (h)	yield (%)
palmitic	2.0	12	49
palmitic	2.0	24	67
palmitic	2.0	36	82
stearic	2.0	36	84
stearic	0	36	80
myristic	2.0	36	79
lauric	2.0	36	78
oleic	2.0	36	83
erucic	2.0	36	81
	0	36	77

Scheme 2. Possible Pathways for the Last Step of TBTU-Promoted Esterification[a]



[a] B = base.

Cholesterol had an R_F that was similar to that of the product. We found it convenient to separate excess cholesterol by acetylation followed by silica gel chromatography, which also allowed separation of the minor amount of α-glycoside. De-*O*-acetylation gave cholesteryl β-D-galactopyranoside (**3**) (Scheme 1).

The critical step is the regioselective addition of fatty acid esters at O-6 of compound **3**. We found that our TBTU-promoted esterification procedure[9] worked well with a variety of fatty acids as shown in Table 2 with yields in the 78 to 84% range. Three changes were made to the conditions previously used for the regioselective acylation of primary hydroxyls in the presence of secondary hydroxyls. The poor solubility of **3** in DMF required a change of solvent, and the reaction was found to proceed well in pyridine. The much longer chain lengths in the fatty acids used here required longer reaction times, and 36 h was found to give reasonable yields. Reactions performed in pyridine without the addition of the more basic tertiary amine, DIEA, gave approximately the same yields as those with DIEA added. The yields obtained are given in Table 2.

This latter observation provides more information about the cause of the selectivity of this TBTU-promoted esterification of primary hydroxyls. The pK_a of the conjugate acid of pyridine in water is 5.25.[19] The smallest pK_a of the nonanomeric secondary hydroxyls of glucose derivatives is about 12.3.[20] That of the primary hydroxyl is about 1.5 to 2 pK_a units larger,[20b,21] approximately 14, and those of galactose are similar.[21] Possible pathways for the last step in the esterification reaction promoted by TBTU are shown in Scheme 2. The amount of alkoxide present with DIEA as the base would be approximately 0.001 [alcohol] because the pK_a of DIEA is about 11.[19] If alkoxide were the nucleophile (lower pathway in Scheme 2), the reaction rate would slow drastically on removal of DIEA because it is approximately 6 pK_a units more basic than pyridine. The absence of marked effects on rates indicates that, in this last step, the base serves only to maintain the appropriate pH and has no role in the pathway that probably includes addition of the primary alcohols to form a tetrahedral intermediate, followed by the rate-determining decomposition of this intermediate (upper pathway in Scheme 2).

Secondary alcohols do not form esters under TBTU-promoted conditions unless the stronger base DBU is present.[9] Because a stronger base would have little effect on the rate of the upper pathway of Scheme 2, the pathway that followed when the base is changed to DBU must involve alkoxide ions. Apparently, primary alcohols are sufficiently less hindered and more nucleophilic to permit their direct addition to the carbonyl group of the benzotriazole-activated ester whereas secondary alcohols need to be activated as alkoxides before they can add.

In summary, short efficient syntheses of a library of one of the two glycolipid antigens against Lyme disease, BbGL1, have been presented that make use of TBTU-promoted primary selective esterification reactions. Overall yields for the three steps from β-D-galactopyranose pentaacetate ranged from 48 to 52% for the different fatty acids.

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Supporting Information Available. Experimental procedures for all syntheses, characterization data of new compounds, and ^{1}H and ^{13}C NMR spectra for all compounds prepared. This information is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.