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Efficient Synthesis of a C-Analogue of the Immunogenic Bacterial Glycolipid BbGL2

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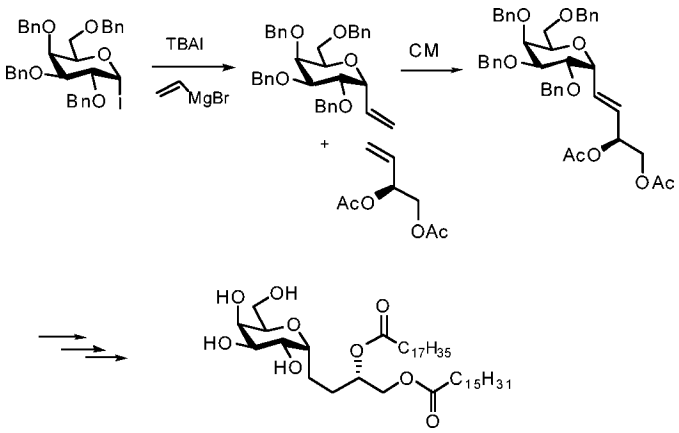
ABSTRACT



Synthesis of a C-analogue of bacterial glycolipid BbGL2 is reported using Grignard reaction of in situ generated *β*-galactosyl iodide and subsequent olefin cross metathesis reaction of *C*-vinyl galactoside as key steps.

Two major glycolipids have been isolated from *Borrelia burgdorferi*, the etiological agent of Lyme disease, which is a multisystemic disorder that affects the skin, nervous system, heart, and joints.[1] The structures of these highly immunoreactive glycolipids have recently been elucidated 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).[2] The major fatty acids were palmitate and oleate. Glycolipids **1** and **2**, being the only antigenic lipid components of *B. burgdorferi*, are looked upon as valuable candidates for diagnosis and potential vaccination for Lyme disease. Moreover, BbGL2 **2** bears a structural resemblance to the powerful immunostimulant α-galactosyl ceramide (α-GalCer) KRN7000 **3**,[3] which is a slightly simplified analogue of glycosphingolipids derived from marine origin (Agelasphins), capable of activating natural killer T cells (NKT cells).[4] The *B. burgdorferi O*-glycosides **1** and **2** have been synthesized;[5] and BbGL2 **2** and its various lipid analogues have been shown to activate mouse and human NKT cells, whereas **1** is inactive.[5b]

Over the years, *C*-glycosides, wherein an acetal linkage is replaced by a methylene group, have been introduced as

(1) Hossain, H.; Wellensiek, H.-J.; Geyer, R.; Lochnit, G. *Biochimie* **2001**, *83*, 683−692.

(2) Ben-Menachem, G.; Kubler-Kielb, J.; Coxon, B.; Yergey, A.; Schneerson, R. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7913−7918.

(3) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Kobayashi, E.; Fukushima, H.; Koezuka, Y. *J. Med. Chem.* **1995**, *38*, 2176−2187.

(4) Kumar, H.; Belperron, A.; Barthold, S. W.; Bockenstedt, L. K. *J. Immunol.* **2000**, *165*, 4797−4801.

(5) (a) Wu, D.; Xing, G.-W.; Poles, M. A.; Horowitz, A.; Kinjo, Y.; Sullivan, B.; Bodmer-Narkevitch, V.; Plattenberg, O.; Kronenberg, M.; Tsuji, M.; Ho, D. D.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 1351−1356. (b) Kinjo, Y.; Tupin, E.; Wu, D.; Fujio, M.; Garcia-Navarro, R.; Benhia, M. R.; Zanonc, D. M.; Ben-Menachem, G.; Ainge, G. D.; Painter, G. F.; Khurana, A.; Hoebe, K.; Behar, S. M.; Beutler, B.; Wilson, I. A.; Tsuji, M.; Sellati, T. J.; Wong, C.-H.; Kronenberg, M. *Nat. Immunol.* **2006**, *7*, 978−986.

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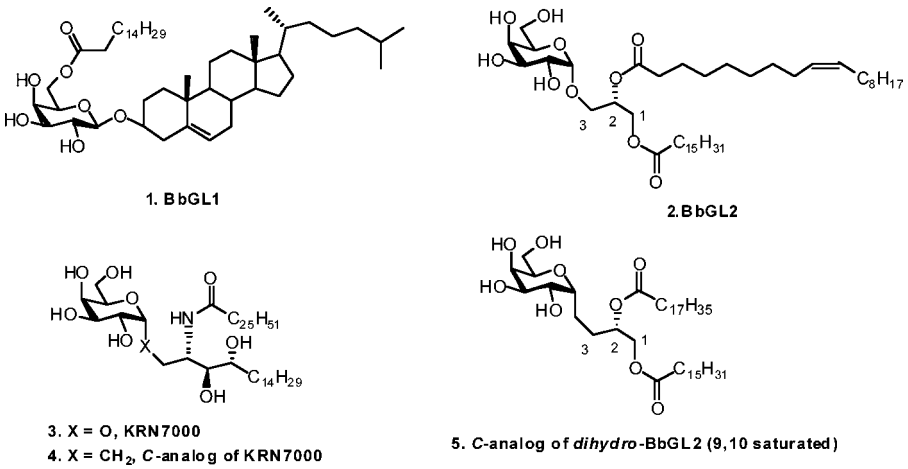
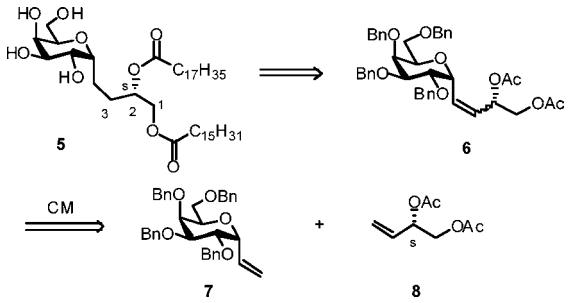


Figure 1. Structures of various biologically significant glycolipids.

stable analogues of *O*-glycosides.[6] Recently, a synthetic *C*-glycoside analogue of KRN7000 (**4**) was shown to exhibit remarkably enhanced activity.[7] In light of these results, C-analogues of the bacterial glycolipids became attractive synthetic targets. Recently, we demonstrated the utility of glycosyl iodides in the synthesis of α-*O*-glycosides[8] and we sought to extend this methodology to the construction of *C*-vinyl glycosides. We envisioned that olefin cross metathesis (CM) of the formed *C*-vinyl galactoside **7** would lead to the target molecule **5**.

Our convergent retro-synthesis of **5** entails two building blocks **7** and **8** as delineated in Scheme 1. The target

Scheme 1. Retrosynthesis of *C*-Glycolipid Dihydro-BbGL2 **5**



molecules can be accessed from di-*O*-acetate **6**, which in turn can be synthesized via CM of *C*-vinyl galactose derivative **7** with (*S*)-3-butene-1,2-diol derived **8**. Franck and co-workers have used a similar strategy, for their second-generation synthesis of the C-analogue of KRN7000, employing ethylene-promoted CM of *C*-vinyl and *C*-propenyl galactosides with vinyl derivatives of phytosphingosine.[9] The Franck protocol offers marked improvement over earlier attempts; however, the preparation of *C*-vinyl galactoside was lengthy and indirect.

The deceptively simple **7** has previously been prepared using two routes either by controlled hydrogenation of the corresponding *C*-ethynyl sugar (30%, five steps) using the Dondoni and Isobe[10] protocol or through Pd-catalyzed double bond isomerization of the corresponding *C*-allyl sugar, generated essentially along Kishi's C-glycosylation route,[11] followed by olefin cross metathesis with ethylene (five steps, 50%). Another indirect method to prepare a related glucoside starts from expensive tri-*O*-benzyl D-glucal and affords the corresponding 2-OH gluco derivative via epoxidation of the double bond and its concomitant syn opening with trivinylaluminum.[12] On the other hand, vinyl Grignard reactions of α-D-glucosyl[13] and α-D-mannosyl[13b] bromides have been reported to proceed with low−moderate yields (30%−45%) and selectivity, generating α/β mixtures that favor the β-isomer. We envisioned that a direct nucleophilic S_N2 displacement of tetra-*O*-benzyl β-galactosyl iodide with vinyl magnesium bromide would be the most direct route to α-vinyl galactoside **8**.

We first streamlined a highly efficient and straightforward route for the preparation of 2,3,4,6-tetra-*O*-benzyl D-galactopyranosyl acetate **10**, amenable to scale-up. As shown in Scheme 2, commercially available α-methyl-D-galactoside

(6) Books and reviews on *C*-glycosides: (a) Levy, D. E.; Tang, C. *The Chemistry of C-Glycosides*; Pergamon: Oxford, 1995. (b) Bertozzi, C. R.; Bednarski, M. D. In *Modern Methods In Carbohydrate Synthesis*; Harwood Academic Publishers: Reading, 1996; pp 316−351. (c) Postema, M. H. D.; Piper, J. L.; Betts, R. L. *Synlett* **2005**, 1345−1358. (d) Yuan, X.; Linhardt, R. J. *Curr. Top. Med. Chem.* **2005**, *5*, 1393−1430.

(7) Yang, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Angew. Chem., Int. Ed.* **2004**, *43*, 3818−3822.

(8) (a) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61−69. (b) Lam, S. N.; Gervay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953−1965. (c) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039−2042. (d) Du, W.; Gervay-Hague, J. *Org. Lett.* **2005**, *7*, 2063−2065.

(9) Chen, G.; Schmieg, J.; Tsuji, M.; Franck, R. W. *Org. Lett.* **2004**, *6*, 4077−4080.

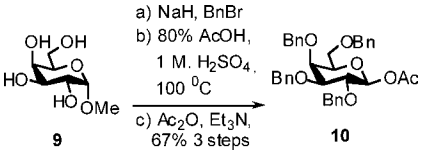
(10) (a) Dondoni, A.; Mariotti, G.; Marra, A. *J. Org. Chem.* **2002**, *67*, 4475−4486. (b) Isobe, M. *J. Synth. Org. Chem. Jpn.* **1994**, *52*, 968−979.

(11) (a) Giannis, A.; Sandhoff, K. *Tetrahedron Lett.* **1985**, *26*, 1479−1482. (b) Lewis, M. D.; Cha, J. K.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 4976−4978.

(12) Rainier, J. D.; Cox, J. M. *Org. Lett.* **2000**, *2*, 2707−2709.

(13) (a) Shulman, M. L.; Shiyan, S. D.; Khorlin, A.Y. *Carbohydr. Res.* **1974**, *33*, 229−235. (b) Jürs, S.; Thiem, J. *Tetrahedron: Asymmetry* **2005**, *16*, 1631−1638.

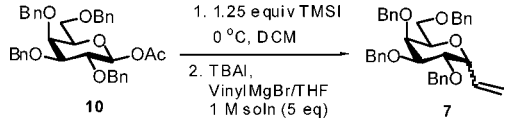
Scheme 2. Efficient Preparation of Anomeric Acetate **10**



9 was per-O-benzylated (NaH/DMF/BnBr). Upon completion of this reaction, DMF was evaporated and the crude product was treated with 1 M H_2SO_4 in 80% AcOH[14] in the same flask and refluxed gently for 8 h. Upon neutralization, the so formed 1-OH hemiacetal was extracted into dichloromethane and acetylated without any further purification using Ac_2O/Et_3N.[15] The crude product, obtained by evaporation, was purified by recrystallization to afford the pure β-isomer (**10**) in 67% overall yield over three steps. Notably, no intermediate column chromatography purifications were necessary. The efficient preparation of starting material enabled further studies of C-nucleophilic additions to glycosyl iodides.

Vinyl Grignard reactions with glycosyl iodides are unprecedented in the literature. We conducted a systematic study of Grignard additions to establish optimum reaction conditions including solvents, temperature, and reagents, as summarized in Table 1. The α-galactosyl iodide is generated

Table 1. Vinyl Grignard Reaction of Galactosyl Iodide



entry	TBAI	solvent	temp	time (h)	α/β (% yield)
1			0 °C to rt	20	1.25:1 (47)
2			rt	20	2:1 (49)
3	1.5	THF	rt	20	3.2:1 (50)
4	1.5	THF	60 °C	3	3.2:1 (32)
5	1.75	benzene	65 °C	1.5	5:1 (60)
6	3.5	benzene	70 °C	1.5	6.2:1 (70)
7	3.5	toluene/benzene (4:1)	100 °C	0.5	7.5:1 (75)
8	3.5	toluene	110 °C	0.5	12:1 (79)

by addition of trimethylsilyliodide (TMSI) into a dichloromethane solution of **10** at 0 °C according to a procedure reported earlier by our laboratory.[8a] The reaction was completed in an hour, and the TMSOAc byproduct was removed by repeated azeotroping with dry benzene. The resulting crude α-galactosyl iodide was subsequently treated with vinyl magnesium bromide (1 M in THF).

We initially tried the reaction without diluting in THF and at 0 °C (entry 1); the requisite *C*-vinyl glycoside **7** was obtained in 47% yield as an α/β mixture with the ratio slightly in favor of the α-isomer. Conducting the same reaction at room temperature resulted in a higher proportion of the α-isomer (α/β = 2:1, 49%, entry 2). This suggested that the reaction was proceeding by a mixed S_N1/S_N2 mechanism owing to the low reactivity of the vinyl Grignard reagent. We believe the β-isomer results from S_N2-like attack on the α-iodide, whereas under conditions of prevailing free iodide ions in solution, the α-iodide anomerizes to the β-iodide, which being more reactive preferentially undergoes attack by the C-nucleophile giving the α-*C*-vinyl compound. This anomerization of glycosyl iodide by iodide ions is favored at higher temperature (thermal effect).[16] We then tried "in situ anomerization" using an external iodide source such as tetrabutylammonium iodide (TBAI) in THF. This reaction gave a higher proportion of α-isomer (3.2:1, 50%) when the Grignard addition was conducted at room temperature (entry 3). On the other hand, conducting the reaction in THF at reflux had an adverse effect on the yield (entry 4), although the selectivity was maintained. At this juncture, we considered the possibility that a nonpolar solvent such as benzene would be a better choice to achieve α-selectivity in the reaction. To this end, we tried slow addition of vinyl Grignard at 65 °C in the presence of 1.75 equiv of TBAI in benzene. As shown in entry 5, the reaction was quenched in 1.5 h and the *C*-galactoside **7** was obtained in 60% yield with an α/β ratio of 5:1. A slight increase in the temperature with a 2-fold increase in TBAI (3.5 equiv, entry 6) resulted in higher yield and selectivity (6.2:1, 65%). This situation was further improved when the reaction was conducted in a toluene/benzene solvent mixture (4:1) at 100 °C affording **7** in 75% isolated yield over two steps with an α/β ratio of 7.5:1 (entry 7). The best results were obtained in toluene at 110 °C; this reaction rapidly offered **7** in 79% over two steps (α/β = 12:1, entry 8). The reaction worked equally well on a 1 g scale, and the α-isomer could be separated upon careful column chromatography. The data of *C*-α **7**[9] and its β-isomer[17] matched well with previously reported values.

With the key synthon *C*-vinyl galactoside **7** in hand, we proceeded to synthesize a simplified *C*-glycoside analogue of BbGL2 (Scheme 3). Our synthetic strategy involved olefin cross metathesis of **7** with **8** as the key step. CM reactions involving vinyl glycosides are reported to proceed in low to modest yields mostly due to steric effects.[18] A recent report by Franck and co-workers showed the first example of

(14) Arya, P.; Barkley, A.; Randell, K. D. *J. Comb. Chem.* **2002**, *4*, 193−198.

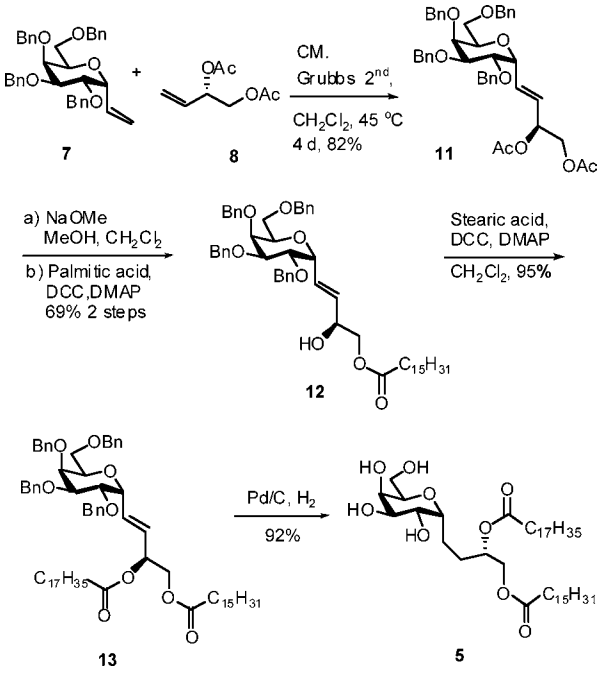
(15) Luo, S.-Y.; Kulkarni, S. S.; Chou, C.-H.; Liao, W.-M.; Hung, S.-C. *J. Org. Chem.* **2006**, *71*, 1226−1229.

(16) El-Badry, M. H.; Gervay-Hague, J. *Tetrahedron Lett.* **2005**, *46*, 6727−6728.

(17) Xie, J.; Durrat, F.; Valery, J.-M. *J. Org. Chem.* **2003**, *68*, 7896−7898.

(18) (a) Roy, R.; Das, S. K.; Dominique, R.; Trono, M. C.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Pure Appl. Chem.* **1999**, *71*, 565−571. (b) Godin, G.; Compain, P.; Martin, O. R. *Org. Lett.* **2003**, *5*, 3269−3272. (c) Dondoni, A.; Giovannini, P. P.; Marra, A. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2380−2388. (d) Nolen, E. G.; Kurish, A. J.; Wong, K. A.; Orlando, M. D. *Tetrahedron Lett.* **2003**, *44*, 2449−2453. (e) Oguri, H.; Sasaki, S.-y.; Oishi, T.; Hirama, M. *Tetrahedron Lett.* **1999**, *40*, 5405−5408. (f) Connon, S. J.; Blechert, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1900−1923.

Scheme 3. Synthesis of the C-Analogue of Dihydro-BbGL2



successful cross metathesis using a *C*-vinyl galactoside under modified conditions of ethylene promotion.[9] However, reaction of the diacetate **8** (3.4 equiv), obtained by acetylation of the corresponding diol, with **7** (1 equiv) under an ethylene atmosphere failed in our hands. The starting materials were recovered after refluxing the reaction in CH_2Cl_2 for 2 days in the presence of Grubbs' catalyst (second generation). We rationalized that the dilution[19] that is required under the ethylene bubbling conditions and/or the presence of ethylene itself may have been detrimental to the success of the reaction. So, we conducted a reaction in the absence of ethylene gas under an argon atmosphere using less dichloromethane. The reaction was refluxed for 4 days with periodic addition of fresh catalyst (25% total). From the onset of the reaction, the formation of the dimer of **8** was observed and its gradual consumption with the increased formation of the product was followed on TLC. Under these conditions, the reaction cleanly afforded the desired olefin **11** in 82% yield as the *E*-isomer. Di-*O*-acetate **11** was deacetylated using NaOMe, and the corresponding crude diol was selectively acylated using palmitic acid, DCC, and DMAP at 0 °C to provide monoacylated **12** as the sole product in 69% overall yield. The remaining secondary hydroxyl was acylated using stearic acid, DCC, and DMAP to produce **13** (95%), which upon hydrogenolysis at 54 psi cleanly afforded the target molecule **5** in 92% yield.

In conclusion, a stable *C*-glycoside analogue of BbGL2 has been prepared in a straightforward manner employing direct C-vinylation of a galactosyl iodide and subsequent olefin cross metathesis as key steps. In this endeavor, we established reaction conditions for the α-stereoselective addition of vinyl Grignard to galactosyl iodide using an "in situ anomerization" process and thereby explored the general principles of the stereoselective C−C bond formation reaction. These general findings should serve as valuable tools for future syntheses of analogues of biologically important *O*-glycolipids. The synthesis of other significant C-analogues of BbGL2 with diverse lipid chains, using the above-established methodology, and their biological evaluation are currently underway in our laboratories.

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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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(19) Hoveyda, H. R.; Vézina, M. *Org. Lett.* **2005**, *7*, 2113−2116.