

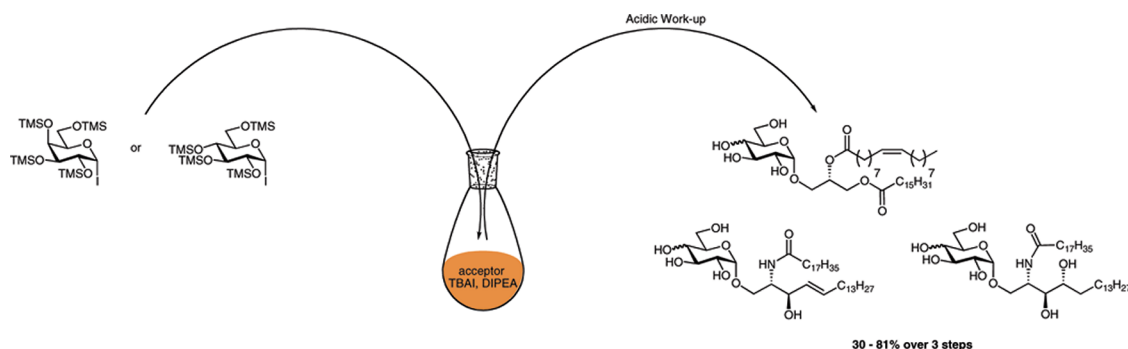
One-Pot Syntheses of Immunostimulatory Glycolipids

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Glycolipids containing α -linked galactosyl and glucosyl moieties have been shown to possess unique immunostimulatory activity creating a need for access to diverse and anomerically pure sources of these compounds for immunological studies. To meet this demand, glycosyl iodides were enlisted in the synthesis of these biologically relevant glycoconjugates. In the first-generation protocol, per-*O*-benzyl galactosyl iodide was efficiently coupled with activated sphingosine acceptors, but fully functionalized ceramides were found to be unreactive. To overcome this obstacle, per-*O*-trimethylsilyl glycosyl iodides were investigated and shown to undergo highly efficient coupling with ceramide and glycerol ester acceptors. Contrary to what has been observed with other donors, we detected little difference between the reactivity of glucosyl and galactosyl iodides. The trimethylsilyl protecting groups play a dual role in activating the donor toward nucleophilic attack while at the same time providing transient protection: the silyl groups are readily removed upon methanolysis. All reactions proceeded with complete acceptor regioselectivity, eliminating the need for additional protecting group manipulations, and the desired α -anomers were formed exclusively. This three-step, one-pot synthetic platform provides rapid access to an important class of immunostimulatory molecules including the first reported synthesis of the glucosyl analogue of the bacterial antigen BbGL-II.

Introduction

In 1993, researchers in Japan reported the isolation and bioactivity studies of the first known α -linked galactosylceramides (α -GalCer).¹ These glycolipids, also known as agelasphins, were extracted from the marine sponge *Agelas mauritianus* and possessed unique antitumor activity. Subsequent structure–activity relationship (SAR) studies led to the identification of the prototypical antigen, KRN7000 (Figure 1). The immunostimulatory effects of the agelasphins attracted considerable attention, as it was especially curious that natural products derived from a marine sponge

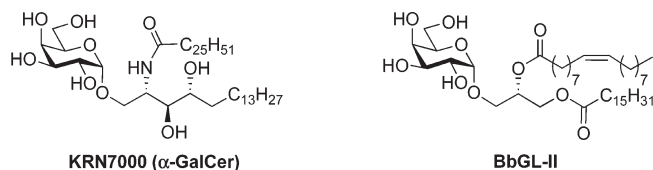
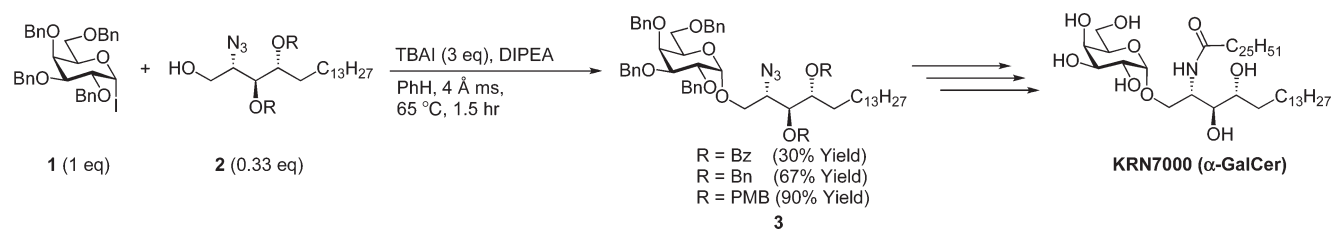


FIGURE 1. Structures of immunostimulatory glycolipids.

could stimulate the immune system. Investigations aimed at identifying other possible sources of α -linked glycolipids commenced. One such study centered on *Borrelia burgdorferi*, which is the causative agent of Lyme disease. Lyme is the most common vector-borne disease in the United States and is a multisystemic disorder that affects the skin, nervous system,

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SCHEME 1. First-Generation Synthesis of KRN7000



heart, muscles, and joints.² Its Gram-negative spirochete is transmitted through the bite of infected ticks and contains the α -linked glycolipid BbGL-II (Figure 1), which exhibits similar antigenic activity to KRN7000. This discovery marked the first example of an NKT cell antigen expressed by a human pathogen and led to the hypothesis that the glycolipids isolated from sponge were of bacterial origin.^{3–7}

Subsequent biological investigations revealed that these glycolipids interact with the lipid binding protein CD1d associated with antigen presenting cells (APC).^{8,9} Once bound, the carbohydrate headgroup of the glycolipid-CD1d complex is presented to a T-cell receptor (TCR) on natural killer T-cells (NKT) which, upon binding, initiates an immunological cascade of events.^{10,11} This immune response includes the release of cytokines mainly composed of the T helper 1 (Th1) cytokine IFN- γ and the T helper 2 (Th2) cytokine IL-4.^{12,13} Th1 responses are important in controlling viral and bacterial infections, as well as tumor metastasis, whereas Th2 cytokine secretion is associated with certain autoimmune diseases such as type-1 diabetes and multiple sclerosis.¹⁴ However, simultaneous production of both Th1 and Th2 cytokines has an antago-

nizing effect resulting from reciprocal inhibition.¹⁵ Therefore, research into the identification of analogues that elicit biased cytokine production is of high interest for therapeutic use.^{16,17}

Since a Th1 response requires prolonged CD1d-glycolipid-TCR binding, the desired polarizing effects may be achieved through modulation of ternary complex longevity.¹⁸ Attempts to tune the stability of the CD1d-glycolipid-TCR construct have focused primarily on modifications to the lipid portion of the antigen. These alterations serve to attenuate complex stability by shifting the spatial orientation of the carbohydrate. Recently it has been shown that variation to the carbohydrate residue may also bias cytokine production. And while it is known that α -mannosyl ceramides (α -ManCer) exhibit no stimulatory activity, α -glucosyl ceramides (α -GlcCer) express similar activity profiles as their α -GalCer epimers.^{11,19} As such, there is a need for access to diverse and anomerically pure sources of α -linked glycolipids to further probe the immunological effects of these antigens. To meet this demand, we recently communicated highly efficient syntheses of bioactive glycolipids with galactose head groups.^{20,21} Herein we report a full account of those studies and extension of the methodology to include glucosyl analogues.

Results and Discussion

Within the past decade, glycosyl iodide chemistry has enjoyed a renaissance largely due to our ability to tame these reactive donors through careful choice of protecting groups. In the context of stereoselective syntheses of α -GalCer analogues, we first reported the coupling of per-*O*-benzyl-protected galactosyl iodide (**1**) with azido phytosphingosine (**2**, Scheme 1).²⁰ TBAI was added to the reaction mixture to promote in situ anomerization of the α -iodide to its more reactive β -anomer. Nucleophilic addition of **2** to the reactive intermediate provided α -galactosyl azido phytosphingosine (**3**) exclusively. In those investigations, we observed a correlation between yield and the protecting groups appended to the nucleophile. For example, when the secondary hydroxyls of **2** were protected with benzoates (Bz), only a 30% yield was observed. Benzyl (Bn) protecting groups, which are electron donating relative to the benzoates, afforded increased product yield (67%), and when *p*-methoxybenzyl (PMB) protecting groups were used, essentially quantitative yields were achieved. Subsequent reduction of the azide and amidation of the resulting amine followed by global deprotection afforded KRN7000 in arguably the most efficient series of reactions reported prior to the date of that publication.

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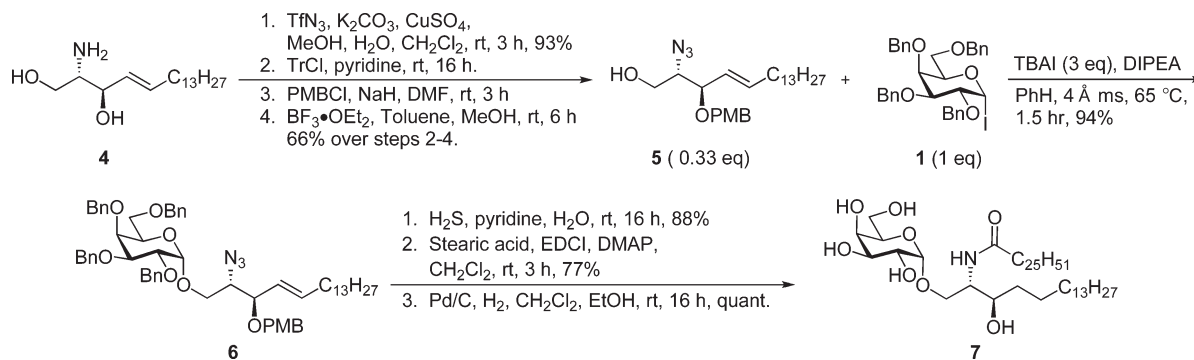
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SCHEME 2. First-Generation Synthesis of Desoxy-KRN7000 (7)



These results cemented our understanding of the electro-
 nic sensitivity of these reactions and the importance of
 matching donor and acceptor reactivity. It is well-known
 that per-*O*-benzylated glycosyl iodides are orders of mag-
 nitude more reactive than their corresponding per-*O*-acety-
 lated analogues. This is evidenced by the fact that per-*O*-
 acetylated glycosyl iodides can be easily purified by column
 chromatography and even crystallized,^{22,23} whereas the ben-
 zyl analogues are far more reactive and can only be isolated
 with great care.²⁴ Likewise, the more electron rich the
 acceptor alcohol, the better suited it is to couple with a
 donor. Conversely, more reactive donors are required for the
 glycosidation of unreactive acceptors. The concept of match-
 ing donor/acceptor reactivity changed our way of thinking
 and resulted in a new direction for our research.

Our ultimate goal was to be able to react fully functionalized
 lipid acceptors with glycosyl iodide donors. We appreciated
 the fact that glycosidation with fully functionalized ceramides
 was notoriously difficult. For example, Sakai and co-workers
 attempted a reaction between per-*O*-benzyl galactosyl fluoride
 and a ceramide using stannous chloride/silver perchlorate
 activation in which only a 23% yield of the desired α -product
 was isolated.²⁵ The main challenge with this strategy is the
 diminished nucleophilicity of the ceramide primary alcohol
 due to intramolecular hydrogen bonding with the amide
 proton.²⁶ We alleviated this problem in our first-generation
 syntheses by masking the amide functionality as an azide;
 however, this process involves many additional steps and
 reduces the overall efficiency. As indicated in Scheme 2, start-
 ing from commercially available sphingosine (4), four steps are
 required to prepare the acceptor for glycosidation. First, the
 amine is converted to an azide through the action of TfN_3 ,
 the primary alcohol is then protected with a trityl group, and
 the secondary alcohol is reacted with *p*-methoxybenzyl chlo-
 ride before removal of the trityl protecting group. Reaction
 of azido sphingosine (5) with 1 is very efficient; however,
 after glycosidation one must essentially reverse the reactions
 required to convert 4 to 5. First the azide is reduced, and then it
 is amidated, and finally the protecting groups are removed to

afford the KRN7000 analogue (7). In the case illustrated, the
 double bond on the lipid was reduced during hydrogenolysis
 although others have reported methods for selectively remov-
 ing benzyl groups while leaving the double bond intact.^{25,27}

Wishing to avoid these protecting group manipulations, we
 turned our attention to the possibility of reacting glycosyl
 iodides with fully functionalized ceramide acceptors (Figure 2).

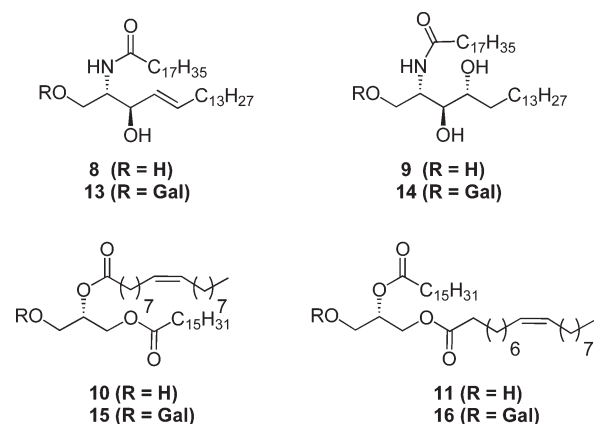


FIGURE 2. Structures of ceramide- and glycerol-containing products and acceptors.

Initial glycosylations between per-*O*-benzyl galactosyl iodide
 (1) and ceramide (8) met with limited success, as only low
 product yields were obtained. However, concurrent studies in
 our lab utilizing per-*O*-trimethylsilyl galactosyl iodide were
 more promising. We found this donor to be orders of mag-
 nitude more reactive than the per-*O*-benzyl analog. Per-*O*-TMS
 glycosides are readily prepared on large scale, and the iodide
 can be generated quantitatively upon reaction with iodotri-
 methylsilane (TMSI).²⁸ An additional benefit associated with
 the use of silyl ether protecting groups is that they can be
 readily removed by methanolysis upon completion of the
 reaction, leaving the glycosidic linkage and olefin unaffected.
 In this manner, the trimethylsilyl functionality serves as a
 transient protecting group.

Our first attempts at developing this methodology focused
 on galactosyl glycolipids.²¹ The syntheses of 13 - 16 began
 with the addition of TMSI to a cooled solution of 12,
 generating per-*O*-TMS galactosyl iodide in situ. The donor

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TABLE 1. Second-Generation Synthesis of Biologically Relevant Glycolipids

entry	acceptor	solvent	conditions	product	$\alpha:\beta$ ratio (yield, %)
1	8	CH ₂ Cl ₂	TBAI (3 equiv), rt, 48 h	13	only α (77)
2	9	CH ₂ Cl ₂	TBAI (3 equiv), rt, 48 h	14	only α (30)
3	9	CH ₂ Cl ₂	TBAI (3 equiv), μ wave, 1.5 h	14	only α (67)
4	10	CH ₂ Cl ₂	TBAI (4 equiv), rt, 24 h	15	only α (81)
5	11	CH ₂ Cl ₂	TBAI (3 equiv), rt, 36 h	16	only α (72)

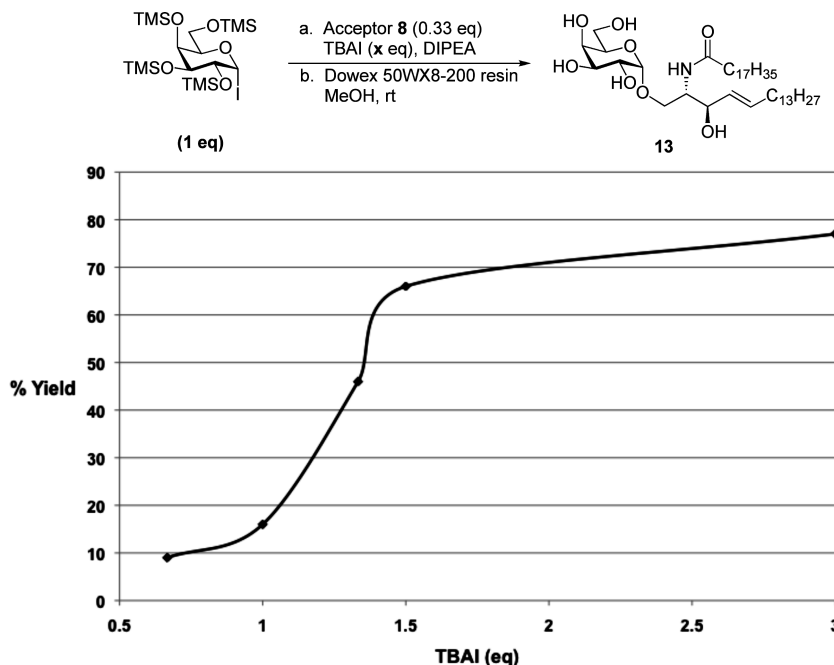


FIGURE 3. TBAI optimization study.

was then added to a mixture containing the acceptor (**8–11**), TBAI, and *N,N*-diisopropylethylamine (DIPEA). After the indicated time, the solvent was evaporated and the residue was subjected to hydrolysis using acidic resin in methanol affording the final products **13–16** (Table 1). Initially, the glycosidation of acceptor **9** proved problematic and at room temperature only low yields (30%, entry 2) were obtained. However, to our delight we found that when the reaction was conducted at 115 °C with 200 W of microwave irradiation, the yield of **14** increased to 67% and the reaction time was reduced from 48 h to 90 min (Table 1, entry 3).

We naturally thought that microwave irradiation would also promote reactions with **8**, but that proved not to be the case. Instead, we observed trans-silylation of the acceptor as evidenced by the disappearance of **8** by thin-layer chromatography (TLC) and its reappearance after methanolysis. Indeed, we were able to confirm that trans-silylation had occurred by isolating and characterizing a silyl-protected acceptor. We hypothesized that TBAI could be promoting desilylation of the sugar generating TMSI, which could in turn react with the acceptor. In an attempt to reduce this process, we explored the possibility of using fewer equiv of TBAI

(Figure 3). These studies indicated that at least 1.5 equiv of TBAI is required for efficient conversion.²⁹ We also attempted microwave irradiation with reduced TBAI and again observed primarily trans-silylation, indicating that room temperature conditions are best for this system.

We next turned our attention to the syntheses of glucose-containing bioactive glycolipids. While there are several published methods for the synthesis of α -GalCer derivatives, there are far fewer reports for the stereoselective synthesis of the corresponding glucosyl analogues. The most commonly employed donor for the synthesis of 1,2-*cis* glucosides has been 2,3,4,6-tetra-*O*-benzyl glucopyranosyl fluoride.³⁰ Typical protocols require the use of toxic reagents (stannous chloride and silver perchlorate), result in α/β product mixtures, and proceed without regioselectivity with respect to the acceptor.³¹ When

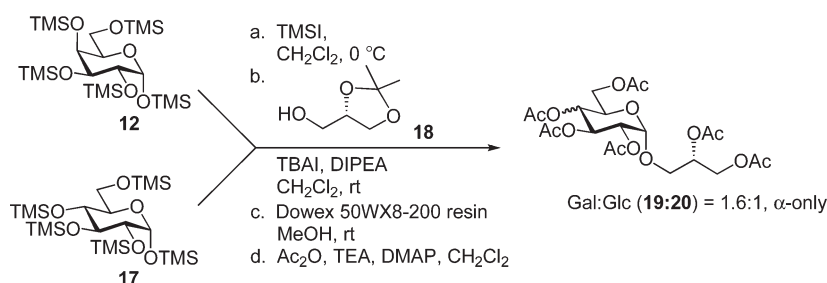
(29) We note that the authors of ref 27 experienced difficulty in removal of TBAI during the synthesis of a galactosyl ceramide in which 3 equiv of TBAI was employed and a yield was not reported. Reduction to 1.5 equiv may help alleviate purification complications.

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TABLE 2. Examples from Competition Studies

Entry	Donor	Catalyst	Acceptor	Product ratio (Gal:Glc)	ref
1		NIS:TfOH	methanol	6.4:1	35
2		DMTST		4:1	36
3		BF3·OEt2	n-octanol	5:1	33

SCHEME 3. Competition Experiment between Per-*O*-TMS Galactosyl and Glucosyl Iodides

applied to the glucosidation of unprotected ceramides, mixtures of both mono and diglycosylated products are formed. Other donors show similar reactivities. For example, Fan and co-workers recently utilized 2,3,4,6-tetra-*O*-benzyl thiophenyl glycopyranoside in the NIS/TfOH promoted glycosidation of a serine-based ceramide. This protocol afforded GlcCer in 46% yield with 2:1 α/β selectivity.³² The reactivity, stereoselectivity, and overall reaction efficiencies are typically reduced with glucosyl donors relative to galactosyl donors. As illustrated by competition studies comparing the reactivity of thioether and trichloroacetimidate donors (Table 2), the relative reactivity ratios were reported to be 6.4:1, 4:1, and 5:1 respectively.^{33–38} A number of theories have been proposed to account for these observations including stereoelectronic effects and torsional strain.^{34,35,38–42}

The key to extending our methodology to glucosyl systems hinged upon the unique reactivity of per-*O*-trimethylsilyl

glycosyl iodides. We were hopeful that the synergistic effects of the TMS groups and the reactive nature of the glycosyl iodide would be sufficient to overcome the relative electron deficiency typically associated with glucosyl donors, resulting in a similar reactivity profile to their galactosyl counterparts. As a first step in our quest to extend this protocol, we initiated a competition study to quantify the relative reactivity of per-*O*-TMS Gal:Glc iodide donors (Scheme 3). Equal amounts of per-*O*-TMS-galactoside (**12**) and per-*O*-TMS-glucoside (**17**) were mixed and TMSI was added to the reaction to generate the respective glycosyl iodide donors in situ.²⁸ Once iodide formation was complete, as evidenced by TLC, the solution was cannulated into a flask containing TBAI, DIPEA, and (*S*)-(+)-1,2-isopropylidene glycerol (**18**), which was chosen as an acceptor because of its relevance to target glucoside **24**. After 36 h, the mixture was subjected to acidic resin-mediated global deprotection followed by acetylation of the resulting free hydroxyls, which allowed for complete characterization of the products. In agreement with our previous reports and based upon ¹H and ¹³C nuclear magnetic resonance (NMR) experiments, only the α -linked glycosides were formed.^{20,21} The Gal:Glc product ratio was found to be 1.6:1, which is much closer than is usually observed with the aforementioned donors. In order to verify the results of the competition experiment, both reactions were conducted separately. In the individual experiments, **19** was obtained in 90% yield and **20** in 79% yield validating the competition experiment.

Next, we focused on the synthesis of the glucosyl analogues **22–24**. We began with the glucosidation of ceramide acceptor **8**. As described previously, per-*O*-TMS glucosyl iodide was transferred into a flask containing the acceptor,

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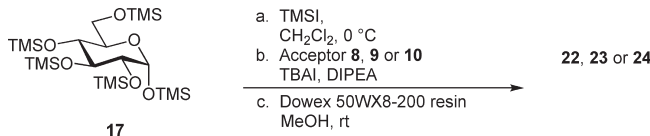
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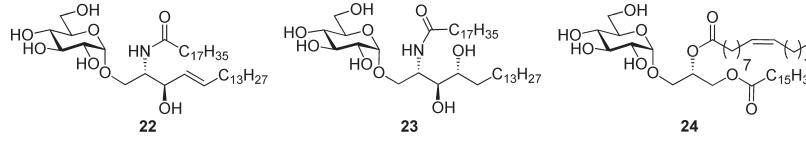
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TABLE 3. Results of the Glucosylation Study



Entry	Acceptor	Solvent	Conditions	Product	α : β ratio (Yield)
1	8	CH ₂ Cl ₂	TBAI (2 eq), rt, 48 h	22	only α (52%)
2	9	CH ₂ Cl ₂	TBAI (3 eq), μ wave, 1.5 h	23	only α (32%)
3	10	CH ₂ Cl ₂	TBAI (2 eq), rt, 48 h	24	only α (58%)



TBAI, and DIPEA. After 48 h, the solvent was removed and the residue was reconstituted in methanol where it was subjected to acidic resin mediated deprotection, affording the desired final product after flash column chromatography. Once again, the reaction progressed in a completely stereoselective fashion, providing **22** in 52% yield from starting material to final product (Table 3, entry 1). The reaction between **17** and glyceride acceptor **10** proceeded in a similar manner, resulting in the first reported synthesis of the glucosyl analogue of the immunological antigen BbGL-II (**24**) in 58% overall yield (Table 3, entry 3). The anomeric configuration of the products was determined using ¹H and ¹³C NMR, and complete assignment was possible using a combination of 1D and 2D homonuclear and heteronuclear NMR experiments. The glucose H–H (³J_{1–2}) coupling constants were all less than *J* = 4 Hz as expected for a 1,2-cis linkage. The presence of only one anomeric carbon, between 96 and 100 ppm, is in agreement with the literature and further indicated the desired α -linkage.

As was the case with our galactose study, initial attempts to incorporate acceptor **9** at room temperature were unsuccessful as none of the desired product (**23**) was recovered. The decreased reactivity is attributed to micelle formation.^{43–45} Various reaction conditions were explored including changes in the solvent, temperature and stoichiometry without success. The only thing we have found thus far to promote this reaction is microwave irradiation. Over the three steps, a 32% yield of **23** was obtained when the mixture was subjected to 200 W and 115 °C for 1.5 h in a sealed microwave vessel (Table 3, entry 2). Although we do not completely understand the mode of action, we have postulated that the microwave conditions increase acceptor accessibility by disrupting ordered lipid arrangement.

Conclusions

We have developed a robust platform for the synthesis of biologically relevant glycolipids based on reacting transiently protected glycosyl iodides with fully functionalized and unprotected acceptors. TBAI was utilized to promote in situ

anomerization of α -glycosyl iodides to the corresponding β -linked reactive intermediates. Our first-generation protocol involved the glycosidation of protected sphingosine or phytosphingosine acceptors with per-*O*-benzyl galactosyl iodide. While this process proceeded with complete α -selectivity, it was only efficient when the acceptor was outfitted with electron-releasing protecting groups. These findings led to the hypothesis that electron-rich glycosyl donors may undergo reaction with fully functionalized ceramide acceptors. Subsequent studies revealed that per-*O*-trimethylsilyl ethers are more reactive than the per-*O*-benzyl counterpart and are indeed sufficiently reactive to glycosylate ceramides. An extra added benefit of the trimethylsilyl protecting group is the ready removal upon methanolysis providing a one-pot method for the syntheses of bioactive α -linked galactosyl ceramides and glycerides. Encouraged by these results, we extended the methodology to encompass traditionally less reactive glycosyl donors, but contrary to our expectation, per-*O*-TMS glycosyl iodide was nearly as reactive as the galactosyl iodide. This methodology provides selectivity for primary alcohols over allylic or secondary alcohols, and no detectable amounts of either the β -glycosides or over glycosylated products were observed. Furthermore, this technology allows for the presence of other functional groups such as esters, amides, and alkenes. This three-step, one-pot protocol is economical and provides rapid access to an important class of immunostimulatory molecules including the first reported synthesis of the glucosyl analogue of the bacterial antigen BbGL-II. We are currently exploring the scope of this methodology for the synthesis of biologically relevant oligosaccharides and glycoconjugates.

Experimental Section

1,2-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*- α -D-galactopyranosyl)-(*S*)-glycerol (19). A solution of per-*O*-trimethylsilyl-D-galactopyranoside (**12**, 1.31 g, 2.42 mmol) in CH₂Cl₂ (3 mL) was cooled to 0 °C followed by the addition of TMSI (532 mg, 2.66 mmol). The reaction mixture was stirred for 20 min at 0 °C then quenched by addition of anhydrous benzene (10 mL) followed by evaporation under reduced pressure to afford the glucosyl iodide as a viscous yellow oil. The iodide was next dissolved in CH₂Cl₂ (3.0 mL) and kept under an argon atmosphere. In a separate flask, TBAI (1.34 g, 3.63 mmol), (*S*)-(+)-1,2-isopropylidene glycerol (**18**, 107 mg, 0.81 mmol), and DIPEA (469 mg, 3.63 mmol) were dissolved in CH₂Cl₂ (5.0 mL), and the mixture was stirred at rt under argon.

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The glycosyl iodide solution was transferred dropwise, via cannula, to the acceptor flask. Once transferred, the reaction mixture was stirred at rt for 36 h. Next, the solvent was removed, and the mixture was reconstituted in MeOH (15 mL) and stirred with Dowex 50WX8-200 ion-exchange resin (1 g) at rt for 4 h. The resin was then removed by filtration, and the solvent was removed in vacuo to afford a brown oil. The crude mixture was acetylated using standard conditions followed by purification using flash column chromatography (CH₂Cl₂/acetone = 97:3, *R_f* = 0.24) to afford **19** as a viscous oil (367 mg, 90%): ¹H NMR (600 MHz, CDCl₃) δ 1.98 (s, 3H), 2.04 (s, 3H), 2.060 (s, 3H), 2.064 (s, 3H), 2.09 (s, 3H), 2.13 (s, 3H), 3.61–3.64 (dd, *J* = 11.4, 5.4 Hz, 1H, H-1a), 3.81–3.83 (dd, *J* = 11.4, 4.2 Hz, 1H, H-1b), 4.08 (d, *J* = 6.6 Hz, 2H, H-6'a, H-6'b), 4.13–4.16 (dd, *J* = 11.4, 5.4 Hz, 1H, H-3a), 4.20 (t, *J* = 6.6 Hz, 1H, H-5'), 4.30–4.33 (dd, *J* = 12.0, 4.2 Hz, 1H, H-3b), 5.08–5.10 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2'), 5.12 (d, *J* = 3.6 Hz, 1H, H-1'), 5.17–5.20 (p, 1H, H-2), 5.30–5.32 (dd, *J* = 10.8, 3.0 Hz, 1H, H-3'), 5.45 (app d, *J* = 3.0 Hz, 1H, H-4'); ¹³C NMR (150 MHz, CDCl₃) δ 20.7, 20.78, 20.81, 21.1, 61.8, 62.3, 66.6, 66.7, 67.6, 68.0, 68.1, 70.2, 96.7, 170.1, 170.30, 170.33, 170.5, 170.59, 170.61; ESI-MS calcd for C₂₁H₃₀O₁₄ [M + Na]⁺ = 529.15, found 529.30.

1,2-Di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*- α -D-glucopyranosyl)-(S)-glycerol (20**).** A solution of per-*O*-trimethylsilyl-D-glucopyranoside (**17**, 1.31 g, 2.42 mmol) in CH₂Cl₂ (3 mL) was cooled to 0 °C followed by the addition of TMSI (532 mg, 2.66 mmol). The reaction mixture was stirred for 20 min at 0 °C then quenched by addition of anhydrous benzene (10 mL) followed by evaporation under reduced pressure to afford the glucosyl iodide as a viscous yellow oil. The iodide was next dissolved in CH₂Cl₂ (3.0 mL) and kept under an argon atmosphere. In a separate flask, TBAI (1.34 g, 3.63 mmol), (S)-(+)-1,2-isopropylidene glycerol (**18**, 107 mg, 0.81 mmol), and DIPEA (469 mg, 3.63 mmol) were dissolved in CH₂Cl₂ (5.0 mL), and the mixture was stirred at rt under argon. The glycosyl iodide solution was transferred dropwise, via cannula, to the acceptor flask. Once transferred, the reaction mixture was stirred at rt for 36 h. Next, the solvent was removed, and the mixture was reconstituted in MeOH (15 mL) and stirred with Dowex 50WX8-200 ion-exchange resin (1 g) at rt for 4 h. The resin was then removed by filtration, and the solvent was removed in vacuo to afford a brown oil. The crude mixture was acetylated using standard conditions followed by purification using flash column chromatography (CH₂Cl₂/acetone = 97:3, *R_f* = 0.24) to afford **20** as a viscous oil (323 mg, 79%): ¹H NMR (600 MHz, CDCl₃) δ 1.99 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.079 (s, 3H), 2.083 (s, 3H), 3.62–3.65 (dd, *J* = 11.4, 5.2 Hz, 1H, H-1a), 3.80–3.83 (dd, *J* = 10.8, 4.2 Hz, 1H, H-1b), 3.97–4.00 (dq, *J* = 10.2, 2.4 Hz, 1H, H-5'), 4.07–4.09 (dd, *J* = 12.6, 2.4 Hz, 1H, H-6'a), 4.14–4.17 (dd, *J* = 12.0, 6.0 Hz, 1H, H-3a), 4.23–4.26 (dd, *J* = 12.6, 4.8 Hz, 1H, H-6'b), 4.30–4.33 (dd, *J* = 12.0, 4.8 Hz, 1H, H-3b), 4.82–4.85 (dd, *J* = 10.2, 4.2 Hz, 1H, H-2'), 5.04 (app t, *J* = 10.2, 9.6 Hz, 1H, H-4'), 5.09 (d, *J* = 4.2 Hz, 1H, H-1'), 5.17–5.20 (p, 1H, H-2), 5.44 (app t, *J* = 10.2, 9.6 Hz, 1H, H-3'); ¹³C NMR (150 MHz, CDCl₃) δ 20.70, 20.73, 20.8, 21.0, 61.9, 62.3, 66.7, 67.7, 68.6, 70.1, 70.2, 70.8, 96.3, 169.7, 170.2, 170.3, 170.4, 170.6, 170.7; ESI-MS calc for C₂₁H₃₀O₁₄ [M + Na]⁺ = 529.15, found 529.30.

1-*O*-Palmitoyl-2-*O*-oleoyl-3-*O*- α -D-glucopyranosyl-*sn*-glycerol (Glc-BbGL-II, **24).** A solution of per-*O*-trimethylsilyl-D-glucopyranoside (**17**, 136 mg, 0.25 mmol) in CH₂Cl₂ (3 mL) was cooled to 0 °C followed by the addition of TMSI (56 mg, 0.28 mmol). The reaction was stirred for 45 min at 0 °C then quenched by addition of anhydrous benzene (10 mL) followed by evaporation under reduced pressure to afford the glucosyl iodide as a viscous yellow oil. The iodide was next dissolved in CH₂Cl₂ (2.0 mL) and kept under an argon atmosphere. In a separate flask, TBAI (186 mg, 0.50 mmol), **10** (50 mg, 0.08 mmol), and DIPEA (65 mg, 0.50 mmol) were dissolved in CH₂Cl₂ (3.0 mL), and the mixture was stirred at rt under argon. The glycosyl iodide solution was transferred dropwise, via

cannula, to the acceptor flask. Once transferred, the reaction mixture was stirred at rt for 48 h. Next, the solvent was removed, and the mixture was reconstituted in MeOH (10 mL) and stirred with Dowex 50WX8-200 ion-exchange resin (0.5 g) at rt for 4 h. The resin was then removed by filtration, and the solvent was removed in vacuo to afford a brown oil which was purified using flash column chromatography (CHCl₃/MeOH = 95:5, *R_f* = 0.36) to afford **24** as a white foam (36 mg, 58%): [α]_D²⁵ +34.4 (*c* = 1.0, CH₂Cl₂); ¹H NMR (600 MHz, CDCl₃) δ 0.87 (t, *J* = 6.6 Hz, 7H), 1.25–1.29 (m, 54H), 1.60 (bs, 4H), 2.01 (q, *J* = 12.0, 6.0 Hz, 4H), 2.31 (dt, *J* = 7.2, 4.2 Hz, 4H), 3.49 (app d, *J* = 13.2 Hz, 1H, H-2'), 3.56–3.60 (m, 3H, H-3a, H-4', H-5'), 3.72 (bt, 1H, H-3'), 3.82 (bs, 3H, H-3b, H-6'a, H-6'b), 4.15 (dd, *J* = 11.4, 6.0 Hz, 1H, H-1a), 4.38 (d, *J* = 9.6 Hz, 1H, H-1b), 4.86 (app s, 1H, H-1'), 5.25 (bt, 1H, H-2), 5.34 (dt, *J* = 10.8, 6.0 Hz, 2H, HC=CH); ¹³C NMR (150 MHz, CDCl₃) δ 14.2, 22.8, 22.9, 25.0, 27.35, 27.38, 29.27, 29.34, 29.41, 29.48, 29.49, 29.53, 29.69, 29.71, 29.83, 29.84, 29.88, 29.91, 29.92, 32.06, 32.08, 34.3, 34.4, 61.7, 62.7, 66.3, 69.9, 72.0, 72.2, 74.3, 99.3, 129.8, 130.2, 173.4, 173.9; ESI-HRMS calcd for C₄₃H₈₀O₁₀ [M – H][–] = 755.5668, found 755.5665.

(2S,3R,4E)-1-*O*-(α -D-Glucopyranosyl)-2-(*N*-octadecanoylamino)octadec-4-ene-1,3-diol (22**).** A solution of per-*O*-trimethylsilyl-D-glucopyranoside (**17**, 162 mg, 0.3 mmol) in CH₂Cl₂ (3 mL) was cooled to 0 °C followed by the addition of TMSI (56 mg, 0.3 mmol). The reaction mixture was stirred for 45 min at 0 °C and then quenched by addition of anhydrous benzene (10 mL) followed by evaporation under reduced pressure to afford the glucosyl iodide as a viscous yellow oil. The iodide was next dissolved in CH₂Cl₂ (2.0 mL) and kept under an argon atmosphere. In a separate flask, TBAI (222 mg, 0.6 mmol), **8** (56 mg, 0.1 mmol), and DIPEA (77 mg, 0.6 mmol) were dissolved in CH₂Cl₂ (3.0 mL) and stirred at rt under argon. The glycosyl iodide solution was transferred dropwise, via cannula, to the acceptor flask. Once transferred, the reaction mixture was stirred at rt for 48 h. Next, the solvent was removed, and the mixture was reconstituted in MeOH (10 mL) and stirred with Dowex 50WX8-200 ion-exchange resin (0.5 g) at rt for 4 h. The resin was then removed by filtration, and the solvent was removed in vacuo to afford a brown oil which was purified using flash column chromatography (CH₂Cl₂/MeOH = 90:10, *R_f* = 0.36). The product (**22**) was obtained as a white foam (37 mg, 52%): [α]_D²⁵ –31.6 (*c* = 1.0, CH₂Cl₂); ¹H NMR (600 MHz, CDCl₃/CD₃OD) δ 0.69 (t, *J* = 7.2 Hz, 8H), 1.07–1.11, m, 66H), 1.16–1.18 (m, 4H), 1.39–1.41 (m, 2H), 1.84 (q, *J* = 7.2 Hz, 2H), 2.01 (t, *J* = 7.2 Hz, 2H), 3.17 (p, *J* = 1.4 Hz, 2H), 3.19 (t, *J* = 9.6 Hz, 1H, H-4'), 3.26 (dd, *J* = 9.6, 4.2 Hz, 1H, H-2'), 3.36 (dq, *J* = 4.2, 2.4 Hz, 1H, H-5'), 3.47 (t, *J* = 9.0 Hz, 1H, H-3'), 3.53 (dd, *J* = 4.2, 1.2 Hz, 1H, H-1a), 3.54 (app t, *J* = 4.8, 3.0 Hz, 1H, H-6'a), 3.58 (app t, *J* = 4.2, 3.0 Hz, 1H, H-6'b), 3.61 (t, *J* = 3.0 Hz, 1H, H-1b), 3.77 (p, *J* = 3.6 Hz, 1H, H-2), 3.91 (app t, *J* = 6.6 Hz, 1H, H-3), 4.63 (d, *J* = 3.6 Hz, 1H, H-1'), 5.26 (dd, *J* = 15.0, 7.2 Hz, 1H, HC=CH), 5.54 (dt, *J* = 15.0, 7.2 Hz, 1H, HC=CH); ¹³C NMR (150 MHz, CDCl₃/CD₃OD) δ 13.8, 22.5, 25.8, 29.10, 29.17, 29.23, 29.28, 29.39, 29.46, 29.47, 29.51, 31.7, 32.2, 36.3, 53.4, 61.3, 67.2, 70.0, 71.86, 71.88, 73.6, 99.3, 128.9, 134.1, 174.5; ESI-HRMS calcd for C₄₂H₈₁NO₈ [M + H]⁺ = 728.6035, found 728.6031.

(2S,3S,4R)-1-*O*-(α -D-Glucopyranosyl)-2-(*N*-octadecanoylamino)octadecane-1,3,4-triol (23**).** The microwave-assisted one-pot glycosylation began with the cooling of a solution of per-*O*-trimethylsilyl-D-glucopyranoside (**17**, 82 mg, 0.15 mmol) in CH₂Cl₂ (3 mL) to 0 °C followed by the addition of TMSI (30 mg, 0.15 mmol). The reaction mixture was stirred for 45 min at 0 °C then quenched by addition of anhydrous benzene (10 mL) followed by evaporation under reduced pressure to afford the glucosyl iodide as a viscous yellow oil. The iodide was next dissolved in CH₂Cl₂ (2.0 mL) and kept under an argon

atmosphere. Next, TBAI (167 mg, 0.45 mmol), **9** (30 mg, 0.05 mmol), and DIPEA (60 mg, 0.45 mmol) were added to a 10 mL microwave vessel containing CH₂Cl₂ (3.0 mL) and stirred at rt under argon. The glycosyl iodide solution was transferred, via cannula, to the sealed microwave reaction vessel, and the vessel was placed into a microwave reactor. The reaction was conducted at 115 °C for 90 min at 200 W. The reaction mixture was transferred to a round-bottomed flask, and the solvent was removed affording a brown viscous oil which was reconstituted in MeOH (10 mL) and stirred with Dowex 50WX8-200 ion-exchange resin (0.5 g) at rt for 4 h. The resin was then removed by filtration, and the solvent was removed in vacuo to afford a brown oil, which was purified using flash column chromatography as described above to afford **23** as a white foam (12 mg, 32%): $[\alpha]_D^{25} -36.8$ ($c = 1.0$, CH₂Cl₂/MeOH, 93:7); ¹H NMR (600 MHz, C₅D₅N) δ 0.98 (t, $J = 6.6$ Hz, 6H), 1.33–1.39 (m, 50H), 1.42–1.53 (m, 6H), 1.75 (m, 1H), 1.91 (m, 2H), 2.37 (m, 1H), 2.52 (t, $J = 7.2$ Hz, 2H), 4.24 (dd, $J = 9.6, 3.6$ Hz, 1H, H-2'), 4.32 (t, $J = 9.6$ Hz, 1H, H-4'), 4.40–4.50 (m, 4H, H-1a, H-6'a, H-4, H-3), 4.52–4.54 (m, 1H, H-5'), 4.58 (dd, $J = 11.4, 1.8$ Hz, 1H, H-6'b), 4.67 (t, $J = 9.0$ Hz, 1H, H-3'), 4.83

(dd, $J = 10.8, 6.0$ Hz, 1H, H-1b), 5.37 (m, 1H, H-2), 5.69 (d, $J = 4.2$ Hz, 1H, H-1'), 8.47 (d, $J = 8.4$ Hz, 1H, N-H); ¹³C NMR (150 MHz, C₅D₅N) δ 14.8, 23.4, 26.85, 26.94, 30.1, 30.2, 30.3, 30.35, 30.41, 30.47, 30.49, 30.50, 30.51, 30.6, 30.9, 32.6, 34.9, 37.3, 51.8, 63.2, 68.6, 72.4, 72.8, 74.0, 75.1, 75.9, 77.2, 101.5, 173.7; ESI-HRMS calc for C₄₂H₈₃NO₉ [M + H]⁺ = 746.6141, found 746.6143.

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Supporting Information Available: ¹H, ¹³C, 2D COSY, and HETCOR NMR spectral data. This material is available free of charge via the Internet at <http://pubs.acs.org>.