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Synthesis and Structure−Activity Relationship Study of Isoglobotrihexosylceramide Analogues

Wenlan Chen,[†] Chengfeng Xia,[‡] Jinhua Wang,[‡] Prakash Thapa,[§] Yusen Li,[§] Janos Nadas,[†] Wenpeng Zhang,[‡] Dapeng Zhou,*[,§] and Peng George Wang*[,†,‡]

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210, Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210, Department of Melanoma Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, Texas 77054

dzhou@mdanderson.org; pgwang@chemistry.ohio-state.edu

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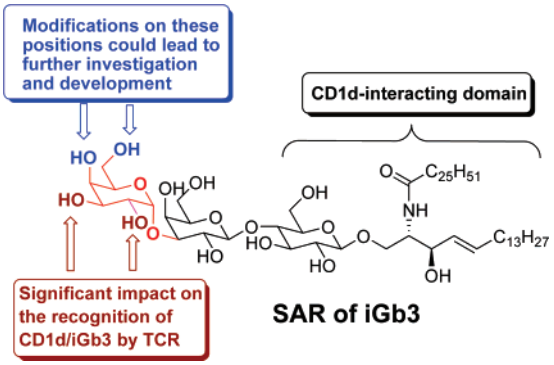

Invariant natural killer T (iNKT) cells are innate T lymphocytes that express T cell receptors binding to exogenous and endogenous glycosphingolpid antigens presented by a nonpolymorphic, non-MHC antigen presenting molecule, CD1d. The endogenous glycosphingolipid metabolite, isoglobotrihexosylceramide (iGb3), is the first known natural ligand for both human and mouse iNKT cells, whose activity has been confirmed in a variety of iNKT cell clones generated by different investigators, representing the majority of the iNKT cell population. The signaling pathway mediated by T cell receptor is largely influenced by the structural variation of glycosphingolpid antigens, leading to multiple and varied biological functions of iNKT cells. In order to investigate the structural requirements behind iGb3 triggered iNKT cell activation, the structure−activity relationship (SAR) of iGb3 needs to be characterized. In this study, iGb3 analogues containing 2‴, 3‴, 4‴ and 6‴ deoxy terminal galactose were synthesized for probing the SAR between iGb3 and TCR. The biological assays on the synthetic iGb3 analogues were performed with use of the murine iNKT cell hybridoma DN32.D3. The results showed that the 2‴ and 3‴ hydroxyl groups of terminal galactose play more important roles for the recognition of iGb3 by TCR; while 4‴ and 6‴ hydroxyl groups were not as crucial for this recognition. These studies might help to understand the general structural requirements for natural endogenous ligands recognized by iNKT cells.

Introduction

A group of specific peptide antigens bound to major histocompatibility complex (MHC) class II or I molecules are recognized by CD4 and CD8 T cells, respectively.[1] In contrast to those peptide antigens, a variety of glycolipids antigens presented by the MHC class I-like CD1d protein are recognized by natural killer T (NKT) cells.[2] The major subset of CD1d restricted NKT cells (also called invariant NKT cells) express semi-invariant Vα T cell receptor (Vα14 in mouse and Vα24 in human).[3] iNKT cells play major roles as a bridging system

* To whom correspondence should be addressed. P.G.W.: phone 614-292-9884, fax 614-688-3106. D.Z.: phone 713-792-3134, fax 713-563-3424.
[†] Department of Chemistry, The Ohio State University.
[‡] Department of Biochemistry, The Ohio State University.
[§] University of Texas M.D. Anderson Cancer Center.

(1) (a) Krummel, M. F.; Davis, M. M. *Curr. Opin. Immunol.* **2002**, *14*, 66−74. (b) Bromley, S. K.; Burack, W. R.; Johnson, K. G.; Somersalo, K.; Sims, T. N.; Sumen, C.; Davis, M. M.; Shaw, A. S.; Allen, P. M.; Dustin, M. L. *Annu. Rev. Immunol.* **2001**, *19*, 375−396. (c) Gao, G. F.; Jakobsen, B. K. *Immunol. Today* **2000**, *21*, 630−636.

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between innate and adaptive immunity.[4] Like natural killer cells, iNKT cells are activated in the first line of immune response within 2 to 6 h upon stimulation to secret proinflammatory T helper 1 (Th1) and immunomodulatory Th2 cytokines and chemokines which allows iNKT cells to regulate a number of disease states in vivo, including malignancy and infection, as well as autoimmune diseases.[5]

Ever since the first discovery of α-galactosylceramides (α-GalCer),[6] it has been verified that α-galactosyl lipids, e.g., KRN7000,[7] can stimulate iNKT cells to produce cytokines like interferon-γ (IFN-γ) and interleukin-4 (IL-4). Nevertheless, α-GalCer is not the endogenous ligand for iNKT cells. In 2004, Zhou et al. discovered that the lysosomal isoglobotrihexosylceramide (iGb3) was an endogenous glycolipid that could stimulate both human and mouse iNKT cells.[8] After Zhou et al.'s initial discovery, iGb3 has been verified by five other laboratories to stimulate a variety of mouse and human NKT clones representing a majority of invariant NKT population.[9] These include human NKT cell lines, and mouse hybridomas Vα14i-DOβ generated in by Gapin's group,[9e] Vα14i-24.8Aβ that express the invariant T cell receptor α (TCRα) and the TCRβ of the NKT clone 24.8.A originally from Brenner's group,[9f] and N38-2C11 and N38-3C generated by Hayakawa's group.[9g] Studies on iGb3 derivatives indicated that the terminal galactose and its α(1,3) configuration might be crucial for recognition.[8,10] Further proof of this was that when the disaccharide glycolipid Galβ1-4Glcβ1-1′ ceramide (same as iGb3 but without the terminal galactose) could not stimulate mouse Vα14 iNKT TCR cells,[11] and the isoglobotetrahexosylceramide (iGb4) could not stimulate either.[8]

Although several CD1d/glycolipid structures have been determined,[12] the structure of the CD1d/glycolipid/TCR ternary complex has become the most pressing target to completely elucidate the SAR between the CD1d presented glycolipid and TCR. This barrier was recently conquered by Borg et al.[13] Most surprisingly, the NKT TCR adopted an acute docking mode that is directly contradicting to previous hypothetical models generated by molecular modeling.[14] The NKT TCR bound approximately parallel to the long axis of the CD1d-antigen-binding cleft, which is distinct from the "diagonal" footprints observed for MHC class I restricted TCRs. More surprisingly, comparison of unliganded NKT TCR to the NKT TCR in the ternary structure did not show drastic change of conformation upon ligand binding. Thus Borg et al. suggested a "lock-and-key" instead of "induced fit" mode of NKT TCR binding. The other striking finding is that only NKT TCRα is contacting the α-GalCer antigen, while the CDR3β of NKT TCRβ is displaced from the antigen binding groove. The 2′, 3′, and 4′ hydroxyl groups of α-GalCer directly bind to CDR3α through hydrogen bondings, thus leading to the recognition by TCR. While it is not clear yet whether the terminal galactose of iGb3 directly binds to CDR3α loop CD1d/α-GalCer/TCR studies showed that the 3′ and 4′ hydroxyl groups of α-GalCer are adjacent to a large positively charged pocket lined by the CDR3β of NKT TCRβ. This positively charged pocket might also provide a possibility to accommodate more bulky sugar head groups such as the trisaccharide head of iGb3.

The orientation and the fine structure of the terminal sugar moiety might play a critical part for TCR binding, as NKT cells recognize iGb3 (α1,3 linked terminal galactose), but not Gb3 (α1,4 linked terminal galactose).[8] Therefore, the replacement of the hydroxyl groups of terminal galactose with nonpolar hydrogens has the possibility to eliminate potential hydrogen bond interactions, consequently resulting in the SAR of these positions (Figure 1).

Results and Discussion

The key component for the SAR study of iGb3 is the terminal deoxy galatosyl moiety that is introduced in the form of the deoxy galactosyl donor **A**. The retrosynthetic strategy of deoxyhydroxyl-iGb3 (dh-iGb3) is illustrated in Scheme 1. In this strategy, phytosphingosine **D** was employed instead of the

(2) (a) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626−1629. (b) Kronenberg, M.; Gapin, L. *Nat. Rev. Immunol.* **2002**, *2*, 557−568. (c) Swann, J.; Crowe, N. Y.; Hayakawa, Y.; Godfrey, D. I.; Smyth, M. J. *Immunol. Cell Biol.* **2004**, *82*, 323−331. (d) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Sato, H.; Kondo, E.; Harada, M.; Koseki, H.; Nakayama, T.; Tanaka, Y.; Taniguchi, M. *Proc. Natl. Acad. Sci.* **1998**, *95*, 5690−5693. (e) Van Kaer, L. *Immunol. Res.* **2004**, *30*, 139−153. (f) Kamada, N.; Iijima, H.; Kimura, K.; Harada, M.; Shimizu, E.; Motohashi, S.; Kawano, T.; Shinkai, H.; Nakayama, T.; Sakai, T.; Brossay, L.; Kronenberg, M.; Taniguchi, M. *Int. Immunol.* **2001**, *13*, 853−861.

(3) (a) Benglagha, K.; Bendelac, A. *Semin Immunol.* **2000**, *12*, 537−542. (b) Taniguchi, M.; Nakayama, T. *Semin Immunol.* **2000**, *12*, 543−550.

(4) Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q.-S.; Delovitch, T. L. *J. Mol. Med.* **2002**, *80*, 290−300.

(5) (a) Smyth, M. J.; Crowe, N. Y.; Hayakawa, Y.; Takeda, K.; Yagita, H.; Godfrey, D. I. *Curr. Opin. Immunol.* **2002**, *14*, 165−171. (b) Serizawa, I.; Koezuka, Y.; Amao, H.; Saito, T. R.; Takahashi, K. W. *Exp. Anim.* **2000**, *49*, 171−180.

(6) (a) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591−2. (b) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771−2784.

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

(8) Zhou, D.; Mattner, J.; Cantu, C., III; Schrantz, N.; Yin, N.; Gao, Y.; Sagiv, Y.; Hudspeth, K.; Wu, Y.-P.; Yamashita, T.; Teneberg, S.; Wang, D.; Proia, R. L.; Levery, S. B.; Savage, P. B.; Teyton, L.; Bendelac, A. *Science* **2004**, *306*, 1786−1789.

(9) (a) Xia, C.; Yao, Q.; Schuemann, J.; Rossy, E.; Chen, W.; Zhu, L.; Zhang, W.; De Libero, G.; Wang, P. G. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2195−2199. (b) Schuemann, J.; Mycko, M. P.; Dellabona, P.; Casorati, G.; MacDonald, H. R. *J. Immunol.* **2006**, *176*, 2064−2068. (c) Speak, A. O.; Salio, M.; Neville, D. C. A.; Fontaine, J.; Priestman, D. A.; Platt, N.; Heare, T.; Butters, T. D.; Dwek, R. A.; Trottein, F.; Exley, M. A.; Cerundolo, V.; Platt, F. M. *Proc. Natl. Acad. Sci.* **2007**, *104*, 5971−5976. (d) Yu, K. O. A.; Im, J. S.; Illarionov, P. A.; Ndonye, R. M.; Howell, A. R.; Besra, G. S.; Porcelli, S. A. *J. Immunol. Methods* **2007**, *323*, 11−23. (e) Scott-Browne, J. P.; Matsuda, J. L.; Mallevaey, T.; White, J.; Borg, N. A.; McCluskey, J.; Rossjohn, J.; Kappler, J.; Marrack, P.; Gapin, L. *Nat. Immunol.* **2007**, *8*, 1105−1113. (f) Behar, S. M.; Podrebarac, T. A.; Roy, C. J.; Wang, C. R.; Brenner, M. B. *J. Immunol.* **1999**, *162*, 161−167. (g) Gui, M.; Li, J.; Wen, L. J.; Hardy, R. R.; Hayakawa, K. *J. Immunol.* **2001**, *167*, 6236−6246.

(10) Xia, C.; Zhou, D.; Liu, C.; Lou, Y.; Yao, Q.; Zhang, W.; Wang, P. G. *Org. Lett.* **2006**, *8*, 5493−5496.

(11) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626−1629.

(12) (a) Zajonc, D. M.; Cantu, C.; Mattner, J.; Zhou, D.; Savage, P. B.; Bendelac, A.; Wilson, I. A.; Teyton, L. *Nat. Immunol.* **2005**, *6*, 810−818. (b) Koch, M.; Stronge, V. S.; Shepherd, D.; Gadola, S. D.; Mathew, B.; Ritter, G.; Fersht, A. R.; Besra, G. S.; Schmidt, R. R.; Jones, E. Y.; Cerundolo, V. *Nat. Immunol.* **2005**, *6*, 819−826. (c) Wu, D.; Zajonc, D. M.; Fujio, M.; Sullivan, B. A.; Kinjo, Y.; Kronenberg, M.; Wilson, I. A.; Wong, C.-H. *Proc. Natl. Acad. Sci.* **2006**, *103*, 3972−3977.

(13) Borg, N. A.; Wun, K. S.; Kjer-Nielsen, L.; Wilce, M. C. J.; Pellicci, D. G.; Koh, R.; Besra, G. S.; Bharadwaj, M.; Godfrey, D. I.; McCluskey, J.; Rossjohn, J. *Nature* **2007**, *448*, 44−49.

(14) (a) Gadola, S. D.; Koch, M.; Marles-Wright, J.; Lissin, N. M.; Shepherd, D.; Matulis, G.; Harlos, K.; Villiger, P. M.; Stuart, D. I.; Jakobsen, B. K.; Cerundolo, V.; Jones, E. Y. *J. Exp. Med.* **2006**, *203*, 699−710. (b) Kjer-Nielsen, L.; Borg, N. A.; Pellicci, D. G.; Beddoe, T.; Kostenko, L.; Clements, C. S.; Williamson, N. A.; Smyth, M. J.; Besra, G. S.; Reid, H. H.; Bharadwaj, M.; Godfrey, D. I.; Rossjohn, J.; McCluskey, J. *J. Exp. Med.* **2006**, *203*, 661−673.

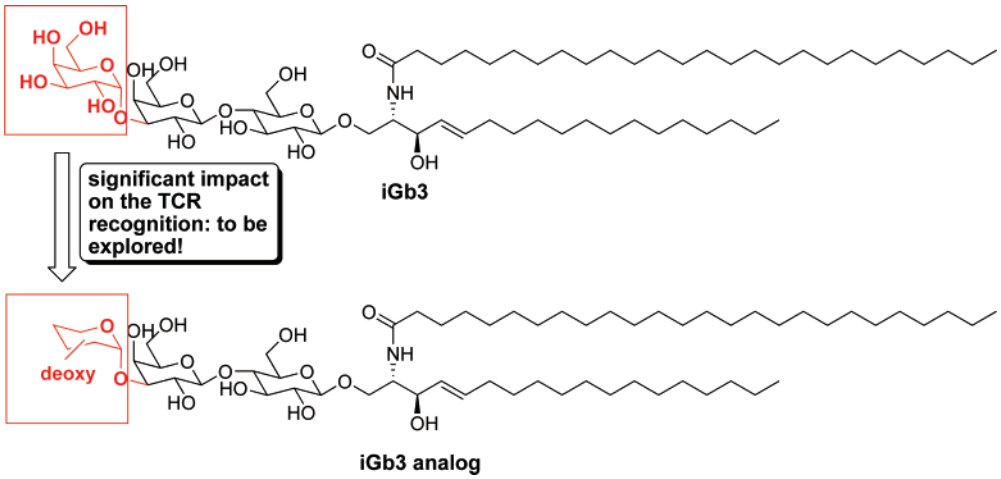
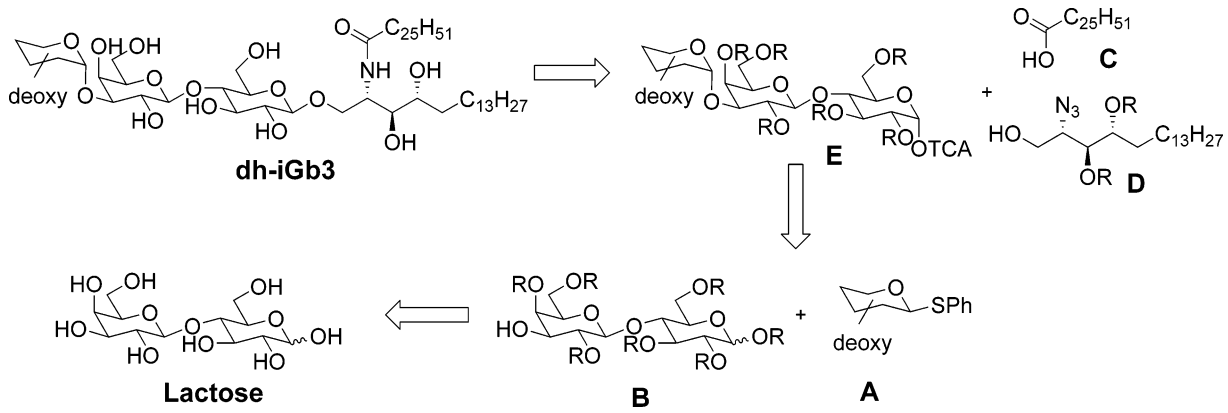
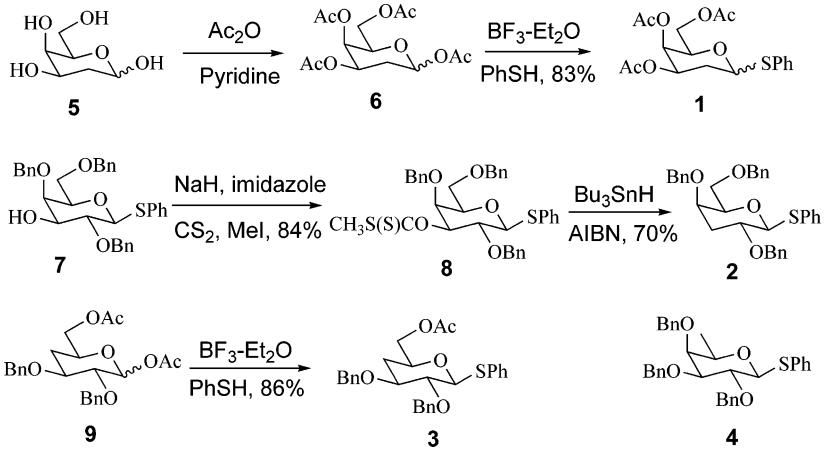


FIGURE 1. The design of the iGb3 analogues for the SAR study.

SCHEME 1. Retrosynthetic Synthesis of dh-iGb3s



SCHEME 2. Synthesis of Terminal Deoxy Donors



original erythro-sphingosine for the synthesis to improve the efficacy in stimulating iNKT cells,[15] without interfering in the study of the terminal sugar moiety. The deoxy trisaccharide donor **E** can be arrived at by the glycosylation of the deoxygalactosyl donor **A** with the disaccharide acceptor **B**, which can be prepared from the commercially available lactose. The glycosylation between **E** and the phytosphingosine acceptor (lipid) **D** followed by an amination reaction with the long chain acid **C** yields the final product dh-iGb3.

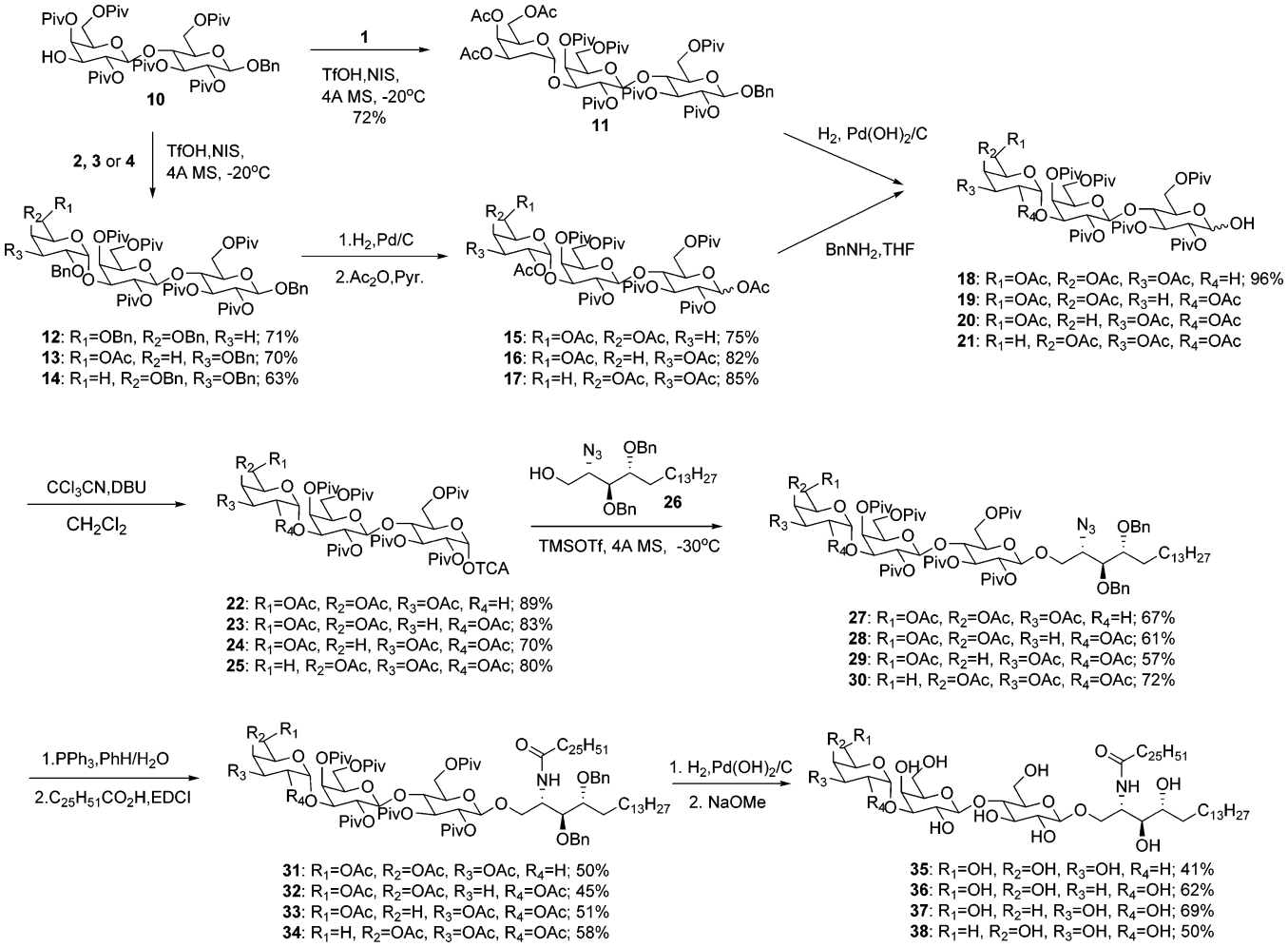
The preparation of deoxy galactosyl donors **1**−**4** is shown in Scheme 2. Peracetylation of the commercially available 2-deoxy galactose **5** followed by the treatment of thiolphenol (PhSH) in the presence of BF_3-Et_2O produced the 2-deoxy donor **1**. The 3-deoxy donor **2** was obtained by deoxygenation of compound **7**[16] through the formation of thiolcarbonate **8** followed by treatment with Bu_3SnH and azobisisobutyronitrile (AIBN).[17] The

(15) Xia, C.; Schümann, J.; Emmanuel, R.; Zhang, Y.; Chen, W.; Zhang, W.; De Libero, G.; Wang, P. G. *J. Med. Chem.* **2007**, *50*, 3489−3496.

(16) Kanie, O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1996**, *37*, 4551−4554.

(17) Lowary, T. L.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *249*, 163−195.

SCHEME 3. Synthesis of dh-iGb3 Analogues



4-deoxy acetylated galactose **9**[18] was treated with PhSH to give the corresponding donor **3**. The 6-deoxy donor **4** was prepared according to the published protocol.[19] Among these four deoxy donors, the benzyl groups were employed as protecting groups for compounds **2**−**4** instead of the acetyl group in donor **1**. This is to eliminate the neighboring group participating effect during the preparation of 3‴, 4‴, and 6‴ deoxy trisaccharides.

The synthesis of these four designed terminal deoxy iGb3 analogues as outlined in Scheme 3 yielded the final product in about 8−10 steps. During the preparation of the deoxy trisaccharide donors, the synthetic route for the 2‴ deoxy trisaccharide donor is slightly different from those for the 3‴, 4‴, and 6‴ deoxy ones. It has been found that, among a variety of promoters, the combination of triflic acid (TfOH) with *N*-iodosuccinimide (NIS) at −20 °C provides a much better α-selectivity for the glycosylations to provide α-galactosyl (α-Gal) derivatives.[20] The glycosylation between 2-deoxy donor **1** and disaccharide acceptor **10**[9a] proceeded smoothly to give trisaccharide **11** by using TfOH as a promoter combining with NIS and 4 Å molecular sieves at −20 °C. From the ^{1}H NMR and ^{1}H−^{1}H COSY spectra of **11**, the α configuration was confirmed by the signal of β-1‴-H at δ 5.28 ppm, which has a small coupling constant with 2‴-H. Hydrogenolysis of the only benzyl group on **11** freed the anomeric hydroxyl group to produce the trisaccharide **18**, which was then converted into the predominant α-configuration trichloroacetimidate donor **22** by using trichloroacetonitrile in the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU).[21]

The preparation of trisaccharide donors **23**−**25** followed a similiar procedure. Glycosylations of acceptor **10** with deoxy galactosyl donors **2**−**4**, under the same reaction conditions as for **11**, resulted in the α-Gal derivatives **12**−**14**. All the benzyl groups of compounds **12**−**14** were removed by hydrogenolysis, and then the following acetylation yielded the fully ester-protected trisaccharides **15**−**17**. Regioselective deprotection of the anomeric acetyl group with use of benzyl amine led to the free anomeric hydroxyl trisaccharides **19**−**21**,[22] which were then subjected to the stereoselective reaction with trichloroacetonitrile to furnish α-trichloroacetimidate donors **23**−**25**.

To improve the reactivity of the lipid acceptor, azido protected phytosphingosine **26** served as the acceptor instead of the ceramide.[15,23] By doing extensive glycosylation experiments

(18) Petrakova, E.; Glaudemans, C. P. J. *Carbohydr. Res.* **1995**, *279*, 133−150.

(19) Janczuk, A. J.; Zhang, W.; Andreana, P. R.; Warrick, J.; Wang, P. G. *Carbohydr. Res.* **2002**, *337*, 1247−1259.

(20) Zhang, W.; Wang, J.; Li, J.; Yu, L.; Wang, P. G. *J. Carbohydr. Chem.* **1999**, *18*, 1009−1017.

(21) Koike, K.; Sugimoto, M.; Sato, S.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 189−208.

(22) Sim, M. M.; Kondo, H.; Wong, C. H. *J. Am. Chem. Soc.* **1993**, *115*, 2260−2267.

(23) Schmidt, R. R.; Zimmerman, P. *Angew. Chem., Int. Ed.* **1986**, *25*, 725−726.

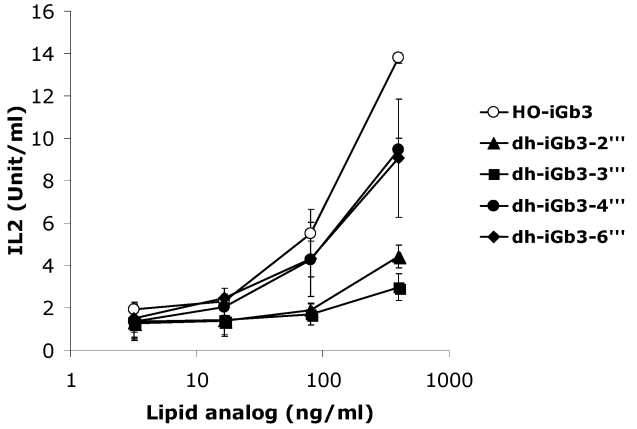


FIGURE 2. Activation of NKT cells with iGb3 analogues. (Data were the average of three independent experiments and analyzed by the SPPS data program. An independent-sample *t*-test was used to measure the statistic significance of the differences between HO-iGb3 and each deoxy-iGb3 analogue at data points of 400 ng/mL. For the difference between 2‴-dh-iGb3 and HO-iGb3, as well as the difference between 3‴-dh-iGb3 and HO-iGb3, the *P* value is lower than 0.001. For the difference between 4‴-dh-iGb3 and HO-iGb3, as well as the difference between 6‴-dh-iGb3 and HO-iGb3, the *P* value is lower than 0.05.)

with a variety of promoters, the highly β-selective glycosylation products with a fairly good yield were obtained when trimethylsilyl trifluoromethanesulfonate (TMSOTf) was used as catalyst at a temperature of −30 °C.[24] Glycosphingosines **27**−**30** were obtained from the glycosylations between trisaccharide donors **22**−**25** and lipid acceptor **26** at −30 °C in the presence of 4 Å molecular sieves and a catalytic amount of TMSOTf. The azido groups on the lipid part were reduced by using triphenylphosphine in benzene with a trace amount of water at 60 °C. The resulting intermediates were treated with cerotic acid and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) in THF to produce the amides **31**−**34**.[25] Finally, the benzyl and all the ester groups were removed by hydrogenolysis and saponification, respectively, to afford the four dh-iGb3s **35**−**38**.

T cell stimulation assays with murine iNKT cell hybridomas were performed as previously described.[8] iGb3 and its analogues were dissolved at 1 mg/mL in DMSO, and stored in −20 °C. The iGb3 analogues in DMSO solution were examined by thin layer chromatography before and after the bioassays to ensure that the glycolipids were not degraded.

Stimulatory activities of iGb3 analogues were studied through the comparison between HO-iGb3[15] and compounds **35**−**38**. After removal of the 2‴ or 3‴ hydroxyl groups, iGb3 analogues (compounds **35** and **36**) showed much weaker stimulatory activity compared to iGb3 with increased concentrations (Figure 2). At 400 ng/mL concentration, 2‴-dh-iGb3 has a 4-fold decrease, while 3‴-dh-iGb3 has a 7-fold decrease. In contrast, 4‴ and 6‴ deoxy iGb3 analogues (compounds **37** and **38**) demonstrated much better stimulation as compared to the 2‴ or 3‴ deoxy ones. The 4‴ and 6‴ deoxy analogues had similar stimulatory activities at the same concentrations. The bioassay experiment was repeated three times giving the same trend each time.

In a recent systemic mutagenesis study on the T cell receptor of invariant NKT cells, Scott-Browne et al. proved that the CDR3α, CDR1α, and CDR2β regions contain the "hot spots" that might be involved in binding to iGb3 and α-GalCer.[9e] Comparing their results to the CD1d/α-GalCer/TCR crystal structure by Borg et al.,[13] we may propose a model that 2‴ and 3‴ OH groups of the terminal α-Gal of iGb3 form hydrogen bonds with CDR3α in a similar way as α-GalCer. In this model, the second Gal of iGb3 might "clash" with the N30 of the CDR1α side chain, as Scott-Browne et al.'s mutagenesis data suggested that the mutation of N30 to A30 may create more space that improve iGb3's fitting to TCR, which is one explanation for the biological assay that N30A mutation of CDR1α surprisingly enhances the T cell hybridoma response to iGb3 antigen; the second model for iGb3-TCR binding is that the terminal α-Gal of iGb3 is accommodated to a "pocket" created by CDR3β.[9e]

All these crystallographic and mutational analyses demonstrated that the orientation of the terminal α-Gal of iGb3 is significant for the recognition of TCR. The 2‴ and 3‴ hydroxyl groups may be critical for this recognition due to the hydrogen bond with CDR loops. On the other hand, the removal of 4‴ or 6‴ hydroxyl groups did not significantly diminish the activity. This finding suggests that the modifications on the 4‴ and 6‴ positions of the terminal α-Gal will be more tolerated than modifications on the 2‴ and 3‴ positions, and proper modifications would lead to further exploration of the activation of NKT cells by CD1d/glycolipid.

Conclusions

In summary, four iGb3 analogues containing terminal deoxy galactose (compounds **35**−**38**) were efficiently synthesized and their activities against mouse NKT cells were investigated. This strategy helped to reveal the SAR of the terminal α-Gal of iGb3. The SAR study pointed out that 2‴ and 3‴ hydroxyl groups should be crucial for the recognition of iGb3 by TCR. It was also suggested that modifications of 4‴ and 6‴ positions of iGb3 with other functional groups do not diminish their stimulatory activity, and would lead to further investigation of the recognition of CD1d/iGb3 by NKT cells. These studies might help to understand the general structural requirements for natural endogenous ligands recognized by NKT cells.

Experimental Section

Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-1-thio-D-galactopyranoside (1). To a solution of acetylated compound **6** (0.84 g, 2.5 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added PhSH (0.31 mL, 3.0 mmol) dropwise, followed by addition of BF_3-Et_2O (0.38 mL, 3.0 mmol). The mixture was allowed to warm up to room temperature slowly. After being stirred for 5 h, the reaction mixture was quenched by saturated $NaHCO_3$ solution (10 mL). The organic layer was washed with water (15 mL) and brine (15 mL) and dried over anhydrous Na_2SO_4. The concentrated residue was purified by flash chromatography (4:1 hexanes/EtOAc) to give 2-deoxy donor **1** (α:β = 2:1; 0.80 g, 83%). ^{1}H NMR (500 MHz, $CDCl_3$) (α-isomer) δ 7.49−7.47 (m, 2H), 7.31−7.24 (m, 3H), 5.76 (d, J = 5.7 Hz, 1H), 5.38 (d, J = 2.7 Hz, 1H), 5.27 (ddd, J = 12.6, 5.0, 3.2 Hz, 1H), 4.69 (t, J = 6.4 Hz, 1H), 4.10−4.09 (m, 2H), 2.49 (ddd, J = 13.0, 13.0, 5.9 Hz, 1H), 2.13 (s, 3H), 2.10−2.04 (m, 1H), 2.00 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 170.3, 170.0, 134.1, 131.7, 129.1, 127.6, 83.8, 67.7, 66.8 (2C), 62.5, 30.8, 20.9, 20.8, 20.7; HRMS calcd for $C_{18}H_{22}O_7SNa$ ([M + Na]$^+$) 405.0984, found 405.0967.

(24) Yao, Q.; Song, J.; Xia, C.; Zhang, W.; Wang, P. G. *Org. Lett.* **2006**, *8*, 911−914.

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

Phenyl 2,4,6-Tri-*O*-benzyl-3-*O*-[(methylthio)thiocarbonyl]-1-thio-β-D-galactopyranoside (8). To a solution of compound **7** (0.42 g, 0.775 mmol) in THF (10 mL) was added NaH (60 mg, 1.55 mmol). After 1 h, imidazole (2.64 mg, 0.039 mmol) was added and the mixture was stirred for 30 min. CS_2 (0.4 mL, 6.59 mmol) was then added, followed by the addition of MeI (0.10 mL, 1.55 mmol) 10 min later. After 3 h, the reaction mixture was diluted with CH_2Cl_2, then washed with saturated $NaHCO_3$ solution and water. The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by flash chromatography (10:1 hexanes/EtOAc) to give the product (0.41 g, 84%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (d, J = 6.2 Hz, 2H), 7.33−7.26 (m, 15H), 7.23−7.18 (m, 3H), 5.85 (dd, J = 9.5, 2.8 Hz, 1H), 4.96 (d, J = 10.3 Hz, 1H), 4.72 (d, J = 9.7 Hz, 1H), 4.69 (d, J = 11.3 Hz, 1H), 4.63 (d, J = 10.5 Hz, 1H), 4.49 (d, J = 11.9 Hz, 1H), 4.45 (d, J = 11.7 Hz, 1H), 4.43 (d, J = 12.0 Hz, 1H), 4.28 (d, J = 2.4 Hz, 1H), 4.12 (d, J = 9.6 Hz, 1H), 3.76 (t, J = 6.2 Hz, 1H), 3.68−3.60 (m, 2H), 2.56 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 215.4, 138.1, 137.8, 133.7, 131.7, 128.9, 128.4, 128.33, 128.30, 128.2, 128.0, 127.9, 127.8, 127.7, 127.3, 87.6, 85.8, 76.9, 75.5(2C), 74.8, 73.6(2C), 68.4, 19.3; HRMS calcd for $C_{35}H_{36}O_5S_3Na$ ([M + Na]$^+$) 655.1623, found 655.1620.

Phenyl 2,4,6-tri-*O*-benzyl-3-deoxy-1-thio-β-D-galactopyranoside (2). To a solution of **8** (0.38 g, 0.60 mmol) in anhydrous toluene was added Bu_3SnH (0.8 mL, 3.0 mmol) and AIBN (0.10 g, 0.60 mmol). The reaction mixture was refluxed for 1 h, then cooled, filtered, and concentrated. The residue was purified by flash chromatography (10:1, hexanes/EtOAc) to give 3-deoxy donor **2** (0.22 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65−7.63 (m, 2H), 7.37−7.25 (m, 18H), 4.78 (d, J = 9.5 Hz, 1H), 4.71 (d, J = 11.5 Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.53 (d, J = 11.1 Hz, 1H), 4.51 (d, J = 12.0 Hz, 1H), 4.48 (d, J = 11.6 Hz, 1H), 4.39 (d, J = 12.0 Hz, 1H), 3.81−3.74 (m, 5H), 2.48 (dt, J = 13.7, 3.4 Hz, 1H), 1.57−1.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.4, 138.3, 138.2, 134.6, 131.6, 128.8, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.75, 127.71, 127.6, 127.0, 89.4, 79.4, 73.6, 72.62, 72.60, 71.8, 71.1, 69.5, 34.1; HRMS calcd for $C_{33}H_{34}O_4SNa$ ([M + Na]$^+$) 549.2076, found 549.2073.

Phenyl 2,3-Di-*O*-benzyl-6-*O*-acetyl-4-deoxy-1-thio-β-D-galactopyranoside (3). To a solution of compound **9** (0.73 g, 1.7 mmol) in CH_2Cl_2 at 0 °C was added PhSH, followed by addition of BF_3−Et_2O. The reaction mixture was stirred overnight. The reaction mixture was quenched by adding saturated $NaHCO_3$ solution. The organic layer was washed with water and brine, then dried over anhydrous Na_2SO_4. The concentrated residue was purified by flash chromatography (8:1 hexanes/EtOAc) to give 4-deoxy donor **3** (0.70 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51−7.50 (m, 2H), 7.43−7.41 (m, 2H), 7.37−7.26 (m, 11H), 5.69 (d, J = 5.2 Hz, 1H), 4.82 (d, J = 11.6 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.75 (d, J = 11.9 Hz, 1H), 4.71 (d, J = 11.6 Hz, 1H), 4.56−4.51 (m, 1H), 4.13 (dd, J = 11.8, 6.6 Hz, 1H), 4.06 (dd, J = 11.7, 3.2 Hz, 1H), 3.89 (ddd, J = 12.6, 10.0, 5.1 Hz, 1H), 3.82 (dd, J = 9.4, 5.1 Hz, 1H), 2.10 (ddd, J = 12.6, 5.0, 2.2 Hz, 1H), 2.00 (s, 3H), 1.54 (dd, J = 11.9, 11.8, 11.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 138.6, 137.9, 134.4, 131.7, 128.9, 128.5, 128.4, 128.0, 127.9, 127.7, 127.6, 127.2, 87.3, 80.0, 75.2, 72.9, 72.5, 66.7, 65.8, 33.4, 20.8; HRMS calcd for $C_{28}H_{30}O_5SNa$ ([M + Na]$^+$) 501.1712, found 501.1713.

General Procedure for the Preparation of Trisaccharides 11−14. Benzyl 3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranoside (11). A suspension of acceptor **10** (430 mg, 0.46 mmol), 2-deoxy donor **1** (160 mg, 0.42 mmol), and 4 Å molecular sieve (500 mg) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min. After the solution was cooled to −30 °C, *N*-iodosuccinamide (100 mg, 0.46 mmol) followed by TfOH (8 μL, 0.10 mmol) was added. The resulting mixture was stirred at −30 °C for 2 h, then was allowed to warm slowly to 0 °C. The mixture was diluted with CH_2Cl_2 (5 mL) and the molecular sieves were filtered. The filtrate was washed with saturated aq $NaHCO_3$ (5 mL), 10% $Na_2S_2O_3$ (5 mL), and brine (5 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel flash chromatography (1:6 EtOAc/hexanes) to furnish trisaccharide **11** (360 mg, 72%) as white foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.24 (m, 5H), 5.41 (d, J = 2.6 Hz, 1H), 5.28 (m, 2H, 1‴-β-H), 5.18 (t, J = 9.5 Hz, 1H), 5.09 (dd, J = 9.3, 8.1 Hz, 1H), 4.96−4.94 (m, 1H), 4.92 (dd, J = 9.2, 8.0 Hz, 1H), 4.80 (d, J = 11.9 Hz, 1H), 4.58 (d, J = 8.2 Hz, 1H), 4.57 (d, J = 8.2 Hz, 1H), 4.55 (d, J = 12.0 Hz, 1H), 4.48 (d, J = 8.1 Hz, 1H), 4.27 (dd, J = 11.9, 5.2 Hz, 1H), 4.12−4.03 (m, 5H), 3.92−3.87 (m, 2H), 3.85 (dd, J = 10.5, 2.9 Hz, 1H), 3.57−3.54 (m, 1H), 2.10 (s, 3H), 2.05−2.00 (m, 1H), 2.04 (s, 3H), 1.84 (s, 3H), 1.55 (dd, J = 12.9, 4.9 Hz, 1H), 1.25 (s, 9H), 1.23 (s, 9H), 1.22 (s, 9H), 1.21 (s, 9H), 1.18 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.6, 177.0, 176.7, 176.2, 170.3, 170.2, 169.8, 136.6, 128.4, 128.0, 127.9, 100.2, 99.4, 93.3, 73.7, 73.5, 72.1, 71.6, 71.5, 71.4, 70.5, 69.7, 67.0, 66.2, 65.8, 64.6, 62.2, 61.8, 61.5, 39.1, 38.9, 38.9, 38.8, 38.7, 38.6, 29.1, 27.3 (2C), 27.1 (2C), 20.7, 20.6 (2C), 14.2; HRMS calcd for $C_{61}H_{92}O_{24}Na$ ([M + Na]$^+$) 1231.5876, found 1231.5864.

Benzyl 2,4,6-Tri-*O*-benzyl-3-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranoside (12). Compound **12** (365 mg, 71%) was obtained from acceptor **10** (390 mg, 0.42 mmol) and donor **2** (200 mg, 0.38 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.23 (m, 18H), 7.15−7.13 (m, 2H), 5.51 (d, J = 2.8 Hz, 1H), 5.19 (t, J = 9.5 Hz, 1H), 5.15 (dd, J = 11.1, 8.2 Hz, 1H), 5.00 (d, J = 2.7 Hz, 1H), 4.91 (dd, J = 9.7, 8.0 Hz, 1H), 4.81 (d, J = 11.9 Hz, 1H), 4.58−4.52 (m, 4H), 4.47 (s, 2H), 4.43 (d, J = 12.1 Hz, 1H), 4.39−4.35 (m, 2H), 4.28−4.25 (m, 2H), 4.02 (d, J = 6.5 Hz, 2H), 3.90 (t, J = 7.1 Hz, 1H), 3.86 (d, J = 9.6 Hz, 1H), 3.83−3.76 (m, 2H), 3.69 (t, J = 6.5 Hz, 1H), 3.62 (s, 1H), 3.58−3.54 (m, 2H), 3.47 (dd, J = 9.2, 5.9 Hz, 1H), 2.01 (ddd, J = 13.0, 3.8, 3.8 Hz, 1H), 1.69 (ddd, J = 13.0, 12.3, 2.3 Hz, 1H), 1.24 (s, 9H), 1.22 (s, 9H), 1.18 (s, 9H), 1.17 (s, 9H), 1.16 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.1, 177.0, 176.7, 175.9, 138.4, 138.3, 138.1, 136.5, 128.34, 128.32, 128.23, 128.20, 127.90, 127.88, 127.7, 127.57, 127.55, 127.51, 127.4, 100.3, 99.2, 95.2, 74.1, 73.7, 73.5, 73.1, 72.8, 71.9, 71.6, 71.3, 71.2, 71.0, 70.7, 70.4, 69.5, 68.4, 66.3, 61.9, 39.0, 38.8, 38.7, 38.66, 38.64, 38.58, 27.3, 27.2, 27.1, 26.3; HRMS calcd for $C_{76}H_{104}O_{21}Na$ ([M + Na]$^+$) 1375.6968, found 1375.6975.

Benzyl 2,3-Di-*O*-benzyl-6-acetyl-4-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranoside (13). Compound **13** (345 mg, 70%) was obtained from acceptor **10** (390 mg, 0.41 mmol) and donor **3** (180 mg, 0.38 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.24 (m, 15H), 5.54 (d, J = 2.8 Hz, 1H), 5.19 (t, J = 9.5 Hz, 1H), 5.09 (dd, J = 10.2, 8.1 Hz, 1H), 4.93 (d, J = 2.9 Hz, 1H), 4.90 (dd, J = 9.6, 7.8 Hz, 1H), 4.83 (d, J = 12.1 Hz, 1H), 4.80 (d, J = 12.0 Hz, 1H), 4.69 (d, J = 11.5 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.58−4.51 (m, 4H), 4.30 (d, J = 8.3 Hz, 1H), 4.28 (dd, J = 11.8, 5.1 Hz, 1H), 4.01−4.00 (m, 4H), 3.92−3.88 (m, 1H), 3.87−3.82 (m, 2H), 3.66 (dd, J = 10.3, 3.2 Hz, 1H), 3.61 (t, J = 6.4 Hz, 1H), 3.53 (ddd, J = 9.6, 5.1, 1.8 Hz, 1H), 3.37 (dd, J = 9.2, 3.2 Hz, 1H), 2.06 (s, 3H), 1.93−1.89 (m, 1H), 1.39 (ddd, J = 12.3, 12.0, 12.0 Hz, 1H), 1.25 (s, 9H), 1.22 (s, 9H), 1.17 (s, 9H), 1.16 (s, 9H), 1.15 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.0, 176.9, 176.7, 175.8, 170.8, 138.7, 138.6, 136.6, 128.4, 128.37, 128.35, 128.0, 127.97, 127.95, 127.8, 127.7, 127.6, 127.5, 100.3, 99.4, 97.8, 80.5, 75.7, 74.9, 73.8, 73.6, 73.5, 72.6, 72.1, 71.6, 71.4, 70.6, 70.5, 67.1, 67.0, 65.8, 62.0, 39.0, 38.9, 38.8, 38.72, 38.71, 38.6, 33.1, 27.34, 27.32, 27.2, 27.14, 27.12, 27.0, 20.8; HRMS calcd for $C_{71}H_{100}O_{22}Na$ ([M + Na]$^+$) 1327.6604, found 1327.6620.

Benzyl 2,3,4-Tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranoside (14). Compound **14** (300 mg, 63%) was obtained from acceptor **10** (360 mg, 0.38 mmol) and donor **4**

(184 mg, 0.35 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41−7.25 (m, 20H), 5.54 (d, J = 2.5 Hz, 1H), 5.18 (t, J = 9.6 Hz, 1H), 5.07 (dd, J = 10.1, 8.3 Hz, 1H), 4.93−4.90 (m, 2H), 4.88−4.87 (m, 1H), 4.85 (s, 2H), 4.80 (d, J = 11.9 Hz, 1H), 4.71 (d, J = 11.8 Hz, 1H), 4.61 (t, J = 10.8 Hz, 2H), 4.58−4.52 (m, 3H), 4.30−4.27 (m, 2H), 3.98−3.93 (m, 3H), 3.86 (dd, J = 10.1, 2.2 Hz, 1H), 3.85 (t, J = 9.4 Hz, 1H), 3.79 (d, J = 6.5 Hz, 1H), 3.58−3.51 (m, 4H), 1.26 (s, 9H), 1.21 (s, 9H), 1.17 (s, 9H), 1.15 (s, 18H), 1.10 (s, 9H), 1.04 (d, J = 6.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.9, 177.8, 177.0, 176.8, 176.7, 175.9, 139.1, 138.7, 138.6, 136.6, 128.44, 128.41, 128.37, 128.33, 128.2, 128.0, 127.97, 127.95, 127.62, 127.61, 127.4, 99.4, 99.1, 79.3, 77.9, 74.8, 74.8, 73.8, 73.5, 73.4, 72.1, 71.7, 71.4, 70.5, 68.0, 67.9, 62.04, 62.01, 39.0, 38.9, 38.8, 38.7, 38.6, 27.3, 27.26, 27.21, 27.15, 27.11, 16.7; HRMS calcd for $C_{76}H_{104}O_{21}Na$ ([M + Na]$^+$) 1375.6968, found 1375.6915.

General Procedure for the Preparation of Trisaccharides 15−17. Acetyl 2,4,6-Tri-*O*-acetyl-3-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-D-glucopyranoside (15). Palladium hydroxide (200 mg, 20 wt %) was added to a solution of trisaccharide **12** (340 mg, 0.25 mmol) in MeOH/EtOAc (2 mL) and the mixture was shaken under 50 psi H_2 for 12 h. The palladium hydroxide was then filtered through a celite pad and concentrated in vacuo to give crude alcohol product. The resulting alcohol was then acetylated to give α,β mixture **15** (220 mg, 2 steps yield 75%). ^{1}H NMR (500 MHz, $CDCl_3$) (α and β mixture; α:β = 1:2.7) δ 6.24 (d, J = 3.8 Hz, 1H), 5.68 (d, J = 8.2 Hz, 1H), 5.47−5.43 (m, 2H), 5.22 (t, J = 9.5 Hz, 1H), 5.15−5.10 (m, 4H), 5.05 (d, J = 3.0 Hz, 2H), 5.00 (s, 4H), 4.93 (t, J = 8.3 Hz, 1H), 4.85 (dd, J = 10.2, 3.9 Hz, 1H), 4.46−4.40 (m, 4H), 4.28 (dd, J = 12.2, 4.4 Hz, 2H), 4.11−4.05 (m, 6H), 4.00−3.95 (m, 4H), 3.92−3.88 (m, 2H), 3.84−3.78 (m, 4H), 3.68−3.66 (m, 1H), 2.10 (s, 3H), 2.06 (s, 6H), 2.02−2.00 (m, 15H), 1.96−1.95 (m, 2H), 1.90−1.84 (m, 2H), 1.21−1.16 (m, 90H), 1.09−1.08 (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.64, 177.58, 177.1, 176.9, 176.86, 176.83, 176.70, 175.95, 175.91, 170.4, 170.1, 169.9, 168.8, 168.7, 100.2, 100.1, 93.9, 93.8, 91.6, 88.6, 74.2, 72.9, 71.9, 71.2, 70.4, 70.1, 67.3, 67.2, 66.4, 65.7, 61.9, 61.5, 61.4, 39.0, 38.9, 38.88, 38.76, 38.72, 38.69, 38.66, 27.4, 27.3, 27.29, 27.26, 27.2, 27.1, 27.0, 26.95, 26.91, 21.2, 20.9, 20.63, 20.62; HRMS calcd for $C_{56}H_{88}O_{25}Na$ ([M + Na]$^+$) 1183.5512, found 1183.5524.

Acetyl 2,3,6-Tri-*O*-acetyl-4-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-D-glucopyranoside (16). Compound **16** (220 mg, 2 steps yield 82%) was obtained from trisaccharide **13** (300 mg, 0.23 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) (α and β mixture; α:β = 1:1.7) δ 6.27 (d, J = 3.8 Hz, 1H), 5.69 (d, J = 8.2 Hz, 1H), 5.49−5.43 (m, 1H), 5.41−5.39 (m, 2H), 5.24 (t, J = 9.3 Hz, 1H), 5.18−5.12 (m, 4H), 4.97−4.93 (m, 5H), 4.87 (dd, J = 10.1, 3.9 Hz, 1H), 4.43−4.35 (m, 6H), 4.14−4.00 (m, 10H), 3.93−3.89 (m, 3H), 3.82−3.77 (m, 4H), 3.68−3.66 (m, 1H), 2.16 (s, 2H), 2.06 (s, 12H), 2.04 (s, 6H), 1.97 (s, 6H), 1.38−1.27 (m, 2H), 1.22−1.18 (m, 90H), 1.11 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.66, 177.61, 177.1, 176.8, 176.79, 176.73, 176.6, 176.0, 175.9, 170.5, 170.1, 169.9, 168.8, 168.6, 100.2, 100.1, 94.4, 94.3, 91.6, 88.5, 74.2, 73.6, 73.5, 72.9, 72.7, 72.0, 71.9, 71.3, 71.1, 70.6, 70.3, 69.7, 69.6, 68.6, 67.6, 65.8, 65.7, 65.2, 65.1, 61.6, 61.5, 61.3, 61.1, 39.0, 38.9, 38.8, 38.7, 38.69, 38.68, 38.65, 38.62, 32.4, 27.3, 27.2, 27.18, 27.15, 27.12, 27.05, 27.03, 26.9, 26.8, 21.1, 20.9, 20.7, 20.67, 20.6; HRMS calcd for $C_{56}H_{88}O_{25}Na$ ([M + Na]$^+$) 1183.5512, found 1183.5490.

Acetyl 2,3,4-Tri-*O*-acetyl-6-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-D-glucopyranoside (17). Compound **17** (146 mg, 2 steps yield 85%) was obtained from trisaccharide **14** (200 mg, 0.15 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) (α and β mixture; α:β = 1:2.1) δ 6.28 (d, J = 3.9 Hz, 1H), 5.70 (d, J = 8.2 Hz, 1H), 5.50−5.46 (m, 1H), 5.41(d, J = 2.6 Hz, 1H), 5.39(d, J = 2.3 Hz, 1H), 5.29−5.21 (m, 6H), 5.18−5.13 (m, 4H), 5.04 (t, J = 3.4 Hz, 1H), 5.01 (t, J = 3.3 Hz, 1H), 4.96 (dd, J = 9.6, 8.4 Hz, 1H), 4.88 (dd, J = 10.1, 3.8 Hz, 1H), 4.41−4.36 (m, 4H), 4.35 (d, J = 8.0 Hz, 1H), 4.07−4.04 (m, 6H), 3.93−3.89 (m, 3H), 3.82−3.80 (m, 2H), 3.73−3.68 (m, 3H), 2.14 (s, 6H), 2.13 (s, 3H), 2.07 (s, 6H), 2.04 (s, 3H), 1.92 (s, 6H), 1.24−1.18 (m, 90H), 1.12−1.11 (m, 24H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.2, 176.85, 176.83, 176.72, 176.71, 176.0, 175.9, 170.5, 170.2, 169.7, 168.8, 168.7, 100.3, 100.2, 95.1, 95.0, 91.7, 75.1, 74.9, 74.2, 73.0, 72.8, 72.2, 72.1, 71.3, 71.1, 70.9, 70.4, 70.0, 69.9, 69.8, 68.6, 67.9, 67.3, 67.2, 65.6, 65.3, 65.2, 61.8, 61.7, 61.6, 61.4, 61.3, 39.1, 39.05, 39.02, 39.0, 38.9, 38.8, 38.78, 38.75, 38.73, 38.6, 27.34, 27.31, 27.28, 27.24, 27.13, 27.09, 27.07, 27.0, 26.9, 21.1, 20.7, 20.6, 20.59, 20.57; HRMS calcd for $C_{56}H_{88}O_{25}Na$ ([M + Na]$^+$) 1183.5512, found 1183.5510.

3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-D-glucopyranose (18). Palladium hydroxide (150 mg, 20 wt %) was added to a solution of trisaccharide **11** (350 mg, 0.29 mmol) in MeOH/EtOAc (4 mL) and the reaction mixture was stirred under hydrogen balloon pressure for 12 h. The reaction mixture was then filtered through a celite pad and concentrated in vacuo to give crude product. Purification on flash chromatography (3:1 hexanes/EtOAc) gave compound **18** (310 mg, 96%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.51 (t, J = 9.8 Hz, 1H), 5.41 (d, J = 2.6 Hz, 1H), 5.35 (d, J = 3.7 Hz, 1H), 5.28−5.26 (m, 2H), 5.27 (d, J = 9.0 Hz, 1H), 5.23 (d, J = 8.9 Hz, 1H), 5.12−5.08 (m, 1H), 5.00−4.96 (m, 1H), 4.73 (d, J = 7.8 Hz, 1H), 4.70 (s, 1H), 4.70−4.69 (m, 1H), 4.68 (d, J = 3.7 Hz, 1H), 4.54−4.50 (m, 1H), 4.53 (d, J = 8.2 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.35−4.29 (m, 2H), 4.14−4.03 (m, 10H), 3.93 (t, J = 9.6 Hz, 1H), 3.91 (t, J = 9.7 Hz, 1H), 3.88−3.83 (m, 4H), 3.59−3.57 (m, 1H), 2.09 (s, 6H), 2.07−2.02 (m, 2H), 2.03 (s, 3H), 2.02 (s, 3H), 1.93 (s, 6H), 1.64−1.59 (m, 1H), 1.56 (dd, J = 12.7, 4.5 Hz, 1H), 1.24 (s, 18H), 1.234 (s, 9H), 1.232 (s, 9H), 1.227 (s, 9H), 1.22 (s, 9H), 1.217 (s, 9H), 1.212 (s, 9H), 1.208 (s, 9H), 1.202 (s, 9H), 1.17 (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.7, 177.8, 177.7, 177.6, 176.8, 176.7, 176.3, 176.2, 170.3 (2C), 170.2, 169.8, 100.0, 95.8, 93.3, 89.9, 73.8, 73.1, 72.0, 71.7, 71.5, 69.6, 68.9, 68.4, 67.0, 66.2, 65.8, 64.6, 62.1 (2C), 61.6, 61.5 (2C), 39.1, 39.0, 38.99, 38.96, 38.9, 38.8, 38.77, 38.68, 29.7, 29.1, 27.37, 27.33, 27.29, 27.26, 27.14, 27.09, 27.0, 20.7, 20.65, 20.61; HRMS calcd for $C_{54}H_{86}O_{24}Na$ ([M + Na]$^+$) 1141.5407, found 1141.5409.

3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-α-D-glucopyranosyl-(1,1)-trichloroacetimide (22). Compound **18** (140 mg, 0.125 mmol) was dissolved in anhydrous CH_2Cl_2 (6 mL). Then CCl_3CN (0.13 mL, 1.25 mmol) and DBU (18 uL, 0.125 mmol) were added, respectively. The reaction mixture was stirred at room temperature for 4 h. The solvent was removed in vacuo and the residue was purified by silica gel flash chromatography (1:4 EtOAc/hexanes) to afford compound **22** (140 mg, 89%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (s, 1H), 6.44 (d, J = 2.6 Hz, 1H), 5.56 (td, J = 9.8, 1.1 Hz, 1H), 5.39 (s, 1H), 5.25 (s, 2H), 5.07 (t, J = 9.3 Hz, 1H), 4.94 (ddd, J = 10.2, 3.7, 1.4 Hz, 1H), 4.90 (m, 1H), 4.47 (d, J = 8.1 Hz, 1H), 4.44 (d, J = 12.1 Hz, 1H), 4.32 (dd, J = 12.2, 4.2 Hz, 1H), 4.13−4.11 (m, 1H), 4.10−4.08 (m, 2H), 4.06 (d, J = 9.1 Hz, 1H), 4.02−4.01 (m, 2H), 3.97 (td, J = 9.7, 1.1 Hz, 1H), 3.86 (d, J = 6.7 Hz, 1H), 3.84−3.82 (m, 1H), 2.08 (s, 3H), 2.02−2.01 (m, 1H), 2.00 (s, 3H), 1.91 (s, 3H), 1.52 (dd, J = 12.7, 4.4 Hz, 1H), 1.20−1.18 (m, 36H), 1.17 (s, 9H), 1.10 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.7, 177.6, 177.54, 177.51, 176.7, 176.2, 170.3, 170.2, 169.8, 160.5, 100.1, 93.3, 92.54, 92.49, 90.8, 72.7, 71.9, 71.8, 71.5, 71.4, 70.1, 69.7, 68.7, 67.0, 66.2, 65.8, 64.5, 62.2, 61.4, 39.1, 38.94, 38.91, 38.8, 38.73, 38.69, 29.0, 27.3, 27.29, 27.24, 27.2, 27.16, 27.12, 27.09, 27.0, 26.9, 20.8, 20.7, 20.6; HRMS calcd for $C_{56}H_{86}Cl_3NO_{24}Na$ ([M + Na]$^+$) 1284.4503, found 1284.4457.

General Procedure for the Preparation of Trisaccharide Donors 23−25. 2,4,6-Tri-*O*-acetyl-3-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-α-D-glucopyranosyl-(1,1)-trichloroacetimide (23). Benzylamine (0.2 mL, 1.8 mmol) was added to a solution of compound **15** (0.21 g, 0.18 mmol) in THF (10 mL). After being stirred for 24 h at room temperature, the reaction mixture was concentrated in

vacuo. The residue was diluted with CH_2Cl_2 (10 mL), then washed with 1 N HCl (5 mL), saturated aq $NaHCO_3$ (5 mL), and brine (5 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and concentrated. The above crude residue was dissolved in anhydrous CH_2Cl_2 (10 mL). Then CCl_3CN (0.2 mL, 2.0 mmol) and DBU (30 uL, 0.20 mmol) were added, respectively. After the solution was stirred at room temperature for 2 h, the solvent was removed in vacuo and the residue was purified by silica gel flash chromatography (1:4 EtOAc/hexanes) to afford compound **23** (190 mg, 2 steps yield 83%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 6.46 (d, J = 3.7 Hz, 1H), 5.57 (t, J = 9.8 Hz, 1H), 5.46 (d, J = 2.2 Hz, 1H), 5.16 (dd, J = 10.3, 7.9 Hz, 1H), 5.08 (d, J = 3.0 Hz, 1H), 5.05−5.01 (m, 2H), 4.96 (dd, J = 10.2, 3.7 Hz, 1H), 4.47 (d, J = 7.9 Hz, 1H), 4.45 (dd, J = 12.3, 2.0 Hz, 1H), 4.34 (dd, J = 12.3, 4.7 Hz, 1H), 4.15−4.05 (m, 4H), 4.02−3.95 (m, 3H), 3.86−3.82 (m, 2H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.00 (ddd, J = 13.5, 4.2, 4.2 Hz, 1H), 1.89 (ddd, J = 13.5, 12.0, 3.1 Hz, 1H), 1.22 (s, 9H), 1.21 (s, 9H), 1.20 (s, 9H), 1.19 (s, 9H), 1.15 (s, 9H), 1.12 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.7, 177.6, 177.5, 176.9, 176.7, 175.9, 170.4, 170.1, 169.9, 160.5, 100.2, 93.8, 92.5, 90.8, 74.1, 72.8, 71.9, 71.5, 70.2, 70.0, 68.7, 67.3, 66.4, 65.6, 61.9, 61.5, 61.3, 60.4, 39.0, 38.9, 38.8, 38.79, 38.7, 38.6, 27.4, 27.3, 27.2, 27.07, 27.04, 26.99, 21.2, 21.0, 20.9, 20.6, 14.2. (This compound is not stable enough to obtain the HRMS spectra.)

2,3,6-Tri-*O*-acetyl-4-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-α-D-glucopyranosyl-(1,1)-trichloroacetimide (24). Compound **24** (160 mg, 2 steps yield 70%) was obtained from **16** (210 mg, 0.18 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 6.47 (d, J = 3.6 Hz, 1H), 5.58 (t, J = 9.8 Hz, 1H), 5.40 (d, J = 2.4 Hz, 1H), 5.19 (d, J = 2.8 Hz, 1H), 5.16 (dd, J = 10.5, 8.2 Hz, 1H), 4.99−4.93 (m, 3H), 4.44 (d, J = 7.9 Hz, 1H), 4.41 (d, J = 3.2 Hz, 2H), 4.15−4.03 (m, 4H), 3.96 (t, J = 9.7 Hz, 3H), 3.83−3.79 (m, 2H), 2.19−2.15 (m, 1H), 2.07 (s, 6H), 1.97 (s, 3H), 1.56 (ddd, J = 12.3, 11.7, 11.7 Hz, 1H), 1.23 (s, 9H), 1.22 (s, 9H), 1.200 (s, 9H), 1.198 (s, 9H), 1.19 (s, 9H), 1.12 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.5, 176.7, 176.6, 176.0, 171.2, 170.6, 170.2, 170.0, 160.6, 100.4, 94.3, 92.6, 90.8, 73.5, 72.9, 72.0, 71.5, 70.7, 70.1, 69.7, 68.7, 67.7, 65.8, 65.2, 61.5, 61.4, 60.4, 39.0, 38.9, 38.8, 38.73, 38.71, 32.5, 30.6, 27.3, 27.2, 27.12, 27.11, 27.09, 27.03, 21.1, 21.0, 20.9, 20.7, 14.2. (This compound is not stable enough to obtain the HRMS.)

2,3,4-Tri-*O*-acetyl-6-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-α-D-glucopyranosyl-(1,1)-trichloroacetimide (25). Compound **25** (210 mg, 2 steps yield 80%) was obtained from **17** (240 mg, 0.21 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 6.48 (d, J = 3.7 Hz, 1H), 5.59 (t, J = 9.8 Hz, 1H), 5.40 (d, J = 2.8 Hz, 1H), 5.24 (dd, J = 10.9, 3.4 Hz, 1H), 5.23 (d, J = 3.2 Hz, 1H), 5.19−5.15 (m, 2H), 5.03 (dd, J = 10.9, 3.2 Hz, 1H), 4.97 (dd, J = 10.3, 3.7 Hz, 1H), 4.43 (dd, J = 12.3, 4.5 Hz, 1H), 4.40 (d, J = 8.1 Hz, 1H), 4.36 (dd, J = 12.2, 1.6 Hz, 1H), 4.17−4.14 (m, 1H), 4.13−4.04 (m, 3H), 3.96 (t, J = 9.7 Hz, 1H), 3.82 (t, J = 6.7 Hz, 1H), 3.73 (dd, J = 10.3, 3.0 Hz, 1H), 2.14 (s, 3H), 2.07 (s, 3H), 1.92 (s, 3H), 1.23 (s, 18H), 1.20 (s, 27H), 1.13−1.12 (m, 12H). (This compound is not stable enough to obtain the ^{13}C NMR and HRMS spectra.)

General Procedure for the Preparation of Glycosphingosines 27−30. 3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-dibenzyloxyoctadecane (27). A suspension of trisaccharide donor **22** (160 mg, 0.12 mmol), sphingosine acceptor **26** (75 mg, 0.15 mmol), and 4 Å molecular sieve (500 mg) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 30 min. After the mixture was cooled to −20 °C, TMSOTf (5 uL, 0.024 mmol) was added. The resulting mixture was stirred at −20 °C for 2 h, and then diluted with CH_2Cl_2 (5 mL). The resulting mixture was quenched by saturated $NaHCO_3$ solution (5 mL) and filtered through a celite pad. The organic layer was separated and washed with brine (5 mL). After being dried over anhydrous Na_2SO_4 and concentrated, the concentrated residue was purified by silica gel flash chromatography (1:4 EtOAc/hexanes) to provide **27** (130 mg, 67%) as clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.26 (m, 10H), 5.66 (d, J = 5.6 Hz, 1H), 5.28 (s, 2H), 5.17 (t, J = 9.6 Hz, 1H), 5.09 (dd, J = 10.1, 8.3 Hz, 1H), 4.98−4.94 (m, 1H), 4.88 (dd, J = 9.6, 8.3 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 4.59 (d, J = 11.4 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.52 (d, J = 11.3 Hz, 1H), 4.51−4.46 (m, 2H), 4.43 (d, J = 7.9 Hz, 1H), 4.26 (dd, J = 11.7, 5.0 Hz, 1H), 4.14−4.03 (m, 2H), 4.00 (dd, J = 10.0, 7.3 Hz, 1H), 3.92−3.86 (m, 2H), 3.84 (dd, J = 10.4, 2.8 Hz, 1H), 3.78 (dd, J = 10.3, 2.8 Hz, 1H), 3.70−3.67 (m, 1H), 3.58−3.57 (m, 2H), 3.52−3.50 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03−2.00 (m, 1H), 1.94 (s, 3H), 1.66−1.47 (m, 3H), 1.55 (dd, J = 12.9, 4.4 Hz, 1H), 1.25−1.20 (m, 26H), 1.23 (s, 9H), 1.22 (s, 9H), 1.20 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H), 1.14 (s, 9H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.6, 177.57, 177.0, 176.6, 176.3, 170.3, 170.2, 169.8, 138.4, 138.0, 128.4, 128.36, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 100.8, 100.1, 93.3, 79.3, 79.2, 73.8, 73.5, 73.4, 72.1, 72.0, 71.6, 71.5, 71.4, 69.7, 69.5, 67.0, 66.2, 65.8, 64.5, 62.2, 61.9, 61.7, 61.4, 39.4, 39.2, 39.1, 39.0, 38.9, 38.74, 38.72, 38.67, 31.9, 30.0, 29.73, 29.69, 29.66, 29.6, 29.57, 29.4, 29.1, 27.3, 27.2, 27.14, 27.11, 27.08, 25.8, 25.4, 22.7, 20.7, 20.64, 20.62, 14.1; HRMS calcd for $C_{86}H_{133}N_3O_{26}Na$ ([M + Na]$^+$) 1646.9075, found 1646.9060.

2,4,6-Tri-*O*-acetyl-3-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-benzyloxyoctadecane (28). Compound **28** (148 mg, 61%) was obtained from donor **23** (184 mg, 0.15 mmol) and acceptor **26** (83 mg, 0.16 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.25 (m, 10H), 5.46 (d, J = 2.6 Hz, 1H), 5.19 (t, J = 9.6 Hz, 1H), 5.13 (dd, J = 10.1, 8.1 Hz, 1H), 5.07 (d, J = 2.9 Hz, 1H), 5.04−5.00 (m, 2H), 4.86 (dd, J = 9.6, 7.9 Hz, 1H), 4.64 (d, J = 11.2 Hz, 1H), 4.58 (d, J = 11.2 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 4.50−4.46 (m, 2H), 4.43 (d, J = 7.8 Hz, 2H), 4.25 (dd, J = 12.0, 5.2 Hz, 1H), 4.10−4.07 (m, 3H), 4.03−3.97 (m, 3H), 3.88 (t, J = 9.6 Hz, 1H), 3.85 (t, J = 7.2 Hz, 1H), 3.82 (dd, J = 10.3, 3.1 Hz, 1H), 3.77 (dd, J = 10.4, 2.8 Hz, 1H), 3.68 (m, 1H), 3.58−3.55 (m, 2H), 3.51 (ddd, J = 9.7, 5.3, 2.0 Hz, 1H), 2.09 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.00 (ddd, J = 13.0, 4.2, 4.2 Hz, 1H), 1.91 (ddd, J = 12.7, 10.5, 3.0 Hz, 1H), 1.66−1.58 (m, 1H), 1.52−1.46 (m, 1H), 1.25−1.23 (m, 24H), 1.22 (s, 9H), 1.190 (s, 9H), 1.194 (s, 9H), 1.180 (s, 9H), 1.179 (s, 9H), 1.14 (s, 9H), 0.88 (t, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.0, 176.9, 176.5, 175.9, 170.4, 170.1, 169.9, 138.3, 137.9, 128.3, 127.9, 127.8, 127.7, 127.6, 100.7, 100.2, 93.9, 79.2, 79.1, 74.4, 73.7, 73.5, 73.4, 71.9, 71.8, 71.5, 71.4, 70.3, 69.4, 67.4, 67.3, 66.4, 65.7, 61.9, 61.8, 61.4, 39.0, 38.84, 38.82, 38.7, 38.6, 31.9, 29.7, 29.65, 29.61, 29.56, 29.52, 29.3, 27.4, 27.3, 27.2, 27.1, 27.08, 27.06, 27.03, 25.3, 22.6, 21.2, 20.9, 20.6, 14.1; HRMS calcd for $C_{86}H_{133}N_3O_{26}Na$ ([M + Na]$^+$) 1646.9075, found 1646.9021.

2,3,6-Tri-*O*-acetyl-4-deoxy-α-D-hexopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-dibenzyloxyoctadecane (29). Compound **29** (130 mg, 57%) was obtained from donor **24** (180 mg, 0.14 mmol) and acceptor **26** (82 mg, 0.16 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.26 (m, 10H), 5.40 (d, J = 2.4 Hz, 1H), 5.21−5.17 (m, 2H), 5.14 (dd, J = 10.2, 8.0 Hz, 1H), 4.97−4.95 (m, 2H), 4.87 (dd, J = 9.6, 7.9 Hz, 1H), 4.65 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 4.56 (d, J = 11.5 Hz, 1H), 4.50 (d, J = 11.5 Hz, 1H), 4.45−4.43 (m, 2H), 4.40 (d, J = 7.9 Hz, 1H), 4.31 (dd, J = 12.1, 4.9 Hz, 1H), 4.14−4.11 (m, 2H), 4.09−4.06 (m, 2H), 4.05−4.01 (m, 1H), 4.00 (d, J = 10.3, 7.3 Hz, 1H), 3.88 (t, J = 9.5 Hz, 1H), 3.82 (t, J = 7.2 Hz, 1H), 3.79 (dd, J = 10.3, 3.1 Hz, 2H), 3.69 (m, 1H), 3.59−3.55 (m, 2H), 3.52 (ddd, J = 9.8, 4.9, 2.0 Hz, 1H), 2.18−2.15 (m, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 1.98 (s, 3H), 1.68−1.47 (m, 3H), 1.39−1.36 (m, 1H), 1.25−1.23 (m, 23H), 1.23 (s, 18H), 1.20 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H), 1.14 (s, 9H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.0, 176.8, 176.6, 176.0, 170.6, 170.1, 170.0, 138.4, 138.0, 128.4, 128.0, 127.8, 127.7, 127.6, 100.8,

100.3, 94.5, 79.3, 79.2, 73.8, 73.7, 73.5, 73.4, 72.0, 71.9, 71.6, 71.4, 70.8, 69.9, 69.5, 67.7, 65.9, 65.3, 65.2, 61.9, 61.8, 61.6, 39.0, 38.9, 38.8, 38.7, 38.6, 32.4, 31.9, 30.0, 29.7, 29.66, 29.61, 29.6, 29.4, 27.4, 27.3, 27.23, 27.21, 27.12, 27.10, 25.4, 22.7, 21.1, 20.9, 20.7, 14.1; HRMS calcd for $C_{86}H_{133}N_3O_{26}Na$ ([M + Na]$^+$) 1646.9075, found 1646.9073.

2,3,4-Tri-*O*-acetyl-6-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-azido-3,4-dibenzyloxyoctadecane (30). Compound **30** (120 mg, 72%) was obtained from donor **25** (130 mg, 0.10 mmol) and acceptor **26** (59 mg, 0.11 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.26 (m, 10H), 5.40 (d, J = 2.6 Hz, 1H), 5.24−5.20 (m, 3H), 5.16−5.12 (m, 2H), 5.03 (dd, J = 11.0, 3.2 Hz, 1H), 4.88 (dd, J = 9.6, 7.9 Hz, 1H), 4.64 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 11.2 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.50 (d, J = 11.5 Hz, 1H), 4.44 (d, J = 7.9 Hz, 1H), 4.40 (dd, J = 12.0, 1.6 Hz, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.32 (dd, J = 12.1, 4.9 Hz, 1H), 4.08−4.06 (m, 3H), 4.01 (dd, J = 10.4, 7.3 Hz, 1H), 3.88 (t, J = 9.5 Hz, 1H), 3.83 (t, J = 6.5 Hz, 1H), 3.79 (dd, J = 10.4, 3.0 Hz, 1H), 3.72 (dd, J = 10.3, 3.1 Hz, 1H), 3.70−3.67 (m, 1H), 3.59−3.56 (m, 2H), 3.52 (ddd, J = 9.8, 4.7, 2.1 Hz, 1H), 2.14 (s, 3H), 2.07 (s, 3H), 1.92 (s, 3H), 1.68−1.47 (m, 3H), 1.39−1.36 (m, 1H), 1.26−1.24 (m, 22H), 1.23 (s, 18H), 1.19 (s, 18H), 1.18 (s, 9H), 1.14 (s, 9H), 1.12 (d, J = 6.2 Hz, 3H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.9, 177.8, 177.0, 176.8, 176.6, 176.0, 170.6, 170.2, 169.8, 138.4, 138.0, 128.4, 128.0, 127.8, 127.7, 127.6, 100.8, 100.2, 95.1, 79.3, 79.2, 75.0, 73.8, 73.5, 73.4, 72.1, 72.0, 71.6, 71.3, 70.9, 68.0, 67.3, 65.6, 65.2, 61.9, 61.6, 39.1, 39.0, 38.9, 38.7, 38.6, 31.9, 30.0, 29.7, 29.69, 29.66, 29.62, 29.5, 29.4, 27.4, 27.3, 27.28, 27.22, 27.16, 27.13, 27.11, 27.08, 27.05, 25.4, 22.7, 21.1, 20.6, 20.5, 14.1; HRMS calcd for $C_{86}H_{133}N_3O_{26}Na$ ([M + Na]$^+$) 1646.9075, found 1646.9076.

General Procedure for the Preparation of Glycoceramides 31−34. 3,4,6-Tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylamino-3,4-dibenzyloxyoctadecane (31). Triphenylphosphine (36 mg, 0.14 mmol) was added to a solution of azide **27** (114 mg, 0.07 mmol) in benzene (10 mL) and water (0.1 mL). The reaction mixture was stirred at 50 °C for 8 h. The solvent was evaporated under reduced pressure and azetroped with benzene (2 × 10 mL), then the residue was dissolved in anhydrous THF (5 mL) and treated with cerotic acid (40 mg, 0.10 mmol) and EDCI (19 mg, 0.10 mmol). After the solution was stirred for 10 h at room temperature, the solvent was evaporated and the residue was partitioned between CH_2Cl_2 (10 mL) and water (5 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After being concentrated, the residue was purified by silica gel flash chromatography (1:5 EtOAc/hexanes) to provide **31** (69 mg, 2 steps yield 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.33 (m, 4H), 7.30−7.27 (m, 6H), 5.65 (d, J = 8.1 Hz, 1H), 5.41 (d, J = 2.4 Hz, 1H), 5.28 (s, 2H), 5.19 (t, J = 9.5 Hz, 1H), 5.08 (dd, J = 10.2, 8.2 Hz, 1H), 4.95 (ddd, J = 12.4, 4.7, 2.8 Hz, 1H), 4.85 (dd, J = 9.6, 7.9 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.60 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.2 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.44 (d, J = 7.8 Hz, 2H), 4.35 (dd, J = 12.2, 1.7 Hz, 1H), 4.28 (dd, J = 12.1, 4.6 Hz, 1H), 4.17−4.13 (m, 2H), 4.12−4.09 (m, 3H), 4.06−4.01 (m, 2H), 3.89 (t, J = 9.5 Hz, 1H), 3.86−3.81 (m, *2*H), 3.78 (dd, J = 7.1, 2.2 Hz, 1H), 3.62−3.59 (m, 1H), 3.52−3.49 (m, 1H), 3.45−3.43 (m, 1H), 2.11 (s, 3H), 2.04 (s, 3H), 2.03−1.91 (m, 2H), 1.94 (s, 3H), 1.65−1.58 (m, 5H), 1.55 (m, 1H), 1.53−1.43 (m, 4H), 1.25−1.24 (m, 64H), 1.23 (s, 9H), 1.22 (s, 9H), 1.20 (s, 9H), 1.19 (s, 9H), 1.15 (s, 9H), 1.13 (s, 9H), 0.88 (t, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.6, 177.5, 177.0, 176.9, 176.3, 172.4, 170.4, 170.3, 169.8, 138.7, 138.6, 128.4, 128.3, 127.8, 127.7, 127.6, 127.5, 101.1, 100.0, 93.3, 80.0, 78.4, 73.7, 73.6, 73.2, 72.0, 71.9, 71.6, 71.4, 71.3, 69.6, 68.9, 67.0, 66.1, 65.8, 64.4, 62.1, 61.7, 61.4, 49.8, 39.1, 39.0, 38.8, 38.7, 38.6, 36.8, 31.9, 29.75, 29.72, 29.7, 29.6, 29.44, 29.40, 27.3, 27.2, 27.1, 27.0, 22.7, 20.8, 20.7, 20.6, 14.1; HRMS calcd for $C_{112}H_{185}NO_{27}Na$ ([M + Na]$^+$) 1999.3032, found 1999.2994.

2,4,6-Tri-*O*-acetyl-3-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylamino-3,4-dibenzyloxyoctadecane (32). Compound **32** (71 mg, 45%) was obtained from **28** (136 mg, 0.08 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35−7.24 (m, 10H), 5.65 (d, J = 8.2 Hz, 1H), 5.45 (d, J = 2.4 Hz, 1H), 5.20 (t, J = 9.5 Hz, 1H), 5.13 (dd, J = 9.6, 7.9 Hz, 1H), 5.07 (d, J = 2.8 Hz, 1H), 5.04−5.00 (m, 2H), 4.84 (dd, J = 9.6, 7.9 Hz, 1H), 4.77 (d, J = 11.3 Hz, 1H), 4.59 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.3 Hz, 1H), 4.48 (d, J = 11.7 Hz, 1H), 4.43 (d, J = 7.8 Hz, 1H), 4.40 (d, J = 7.9 Hz, 1H), 4.33 (dd, J = 12.1, 1.8 Hz, 1H), 4.27 (dd, J = 12.1, 4.8 Hz, 1H), 4.15−4.13 (m, 2H), 4.11−4.05 (m, 3H), 4.02−3.96 (m, 2H), 3.87 (t, J = 9.5 Hz, 1H), 3.84−3.77 (m, 3H), 3.61−3.60 (m, 1H), 3.53−3.49 (m, 1H), 3.45−3.43 (m, 1H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.02−1.98 (m, 2H), 1.96−1.88 (m, 2H), 1.66−1.58 (m, 3H), 1.51−1.43 (m, 3H), 1.24−1.23 (m, 66H), 1.22 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H), 1.17 (s, 9H), 1.15 (s, 9H), 1.13 (s, 9H), 0.87 (t, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.7, 177.5, 176.9, 176.88, 176.83, 175.9, 172.4, 170.4, 170.1, 169.9, 138.7, 138.6, 128.3, 128.2, 127.7, 127.6, 127.55, 127.51, 101.0, 100.1, 93.9, 79.9, 78.4, 74.4, 73.7, 73.5, 73.3, 71.9, 71.8, 71.5, 71.2, 70.2, 68.8, 67.4, 67.2, 66.4, 65.6, 61.9, 61.8, 61.4, 49.8, 39.0, 38.8, 38.84, 38.78, 38.76, 38.68, 38.64, 36.7, 31.9, 29.8, 29.7, 29.69, 29.67, 29.62, 29.4, 29.34, 29.32, 27.4, 27.3, 27.2, 27.1, 27.08, 27.04, 26.1, 25.6, 22.7, 21.2, 20.9, 20.6, 14.1; HRMS calcd for $C_{112}H_{185}NO_{27}Na$ ([M + Na]$^+$) 1999.3032, found 1999.3079.

2,3,6-Tri-*O*-acetyl-4-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylamino-3,4-dibenzyloxyoctadecane (33). Compound **33** (80 mg, 51%) was obtained from **29** (130 mg, 0.08 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.25 (m, 10H), 5.66 (d, J = 8.4 Hz, 1H), 5.39 (d, J = 2.2 Hz, 1H), 5.20 (t, J = 9.5 Hz, 1H), 5.19 (s, 1H), 5.13 (dd, J = 10.1, 8.1 Hz, 1H), 4.98 (br, 2H), 4.85 (dd, J = 9.6, 8.0 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.60 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.2 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.44 (d, J = 7.8 Hz, 1H), 4.37 (d, J = 7.8 Hz, 1H), 4.35 (dd, J = 12.1, 4.2 Hz, 1H), 4.27 (dd, J = 12.1, 1.4 Hz, 1H), 4.18−4.00 (m, 7H), 3.87 (t, J = 9.6 Hz, 1H), 3.81−3.76 (m, 3H), 3.60 (dd, J = 9.6, 3.2 Hz, 1H), 3.51 (m, 1H), 3.44 (dt, J = 8.4, 2.7 Hz, 1H), 2.16 (m, 1H), 2.08 (s, 3H), 2.07 (s, 3H), 1.98 (s, 3H), 1.67−1.45 (m, 10H), 1.25−1.24 (m, 65H), 1.23 (s, 9H), 1.22 (s, 9H), 1.19 (s, 9H), 1.18 (s, 9H), 1.15 (s, 9H), 1.14 (s, 9H), 0.87 (t, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.6, 177.0, 176.8, 176.7, 176.0, 172.4, 170.6, 170.1, 170.0, 138.7, 138.6, 128.3, 128.2, 127.8, 127.6, 127.57, 127.53, 101.1, 100.2, 94.4, 80.0, 78.3, 73.7, 73.4, 73.3, 71.9, 71.6, 70.7, 67.6, 65.8, 65.1, 65.0, 61.5, 49.7, 39.0, 38.9, 38.8, 38.77, 38.68, 38.65, 32.4, 31.9, 29.8, 29.7, 29.68, 29.63, 29.5, 29.4, 29.35, 29.33, 27.3, 27.2, 27.16, 27.15, 27.08, 27.06, 26.1, 25.6, 22.7, 20.7, 14.1; HRMS calcd for $C_{112}H_{185}NO_{27}Na$ ([M + Na]$^+$) 1999.3032, found 1999.3038.

2,3,4-Tri-*O*-acetyl-6-deoxy-α-D-galactopyranosyl-(1,3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl-(1,4)-2,3,6-tri-*O*-pivaloyl-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylamino-3,4-dibenzyloxyoctadecane (34). Compound **34** (80 mg, 58%) was obtained from compound **30** (120 mg, 0.07 mmol). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33−7.25 (m, 10H), 5.64 (d, J = 8.3 Hz, 1H), 5.38 (d, J = 2.6 Hz, 1H), 5.23−5.19 (m, 3H), 5.15−5.12 (m, 2H), 5.02 (dd, J = 10.9, 3.2 Hz, 1H), 4.85 (dd, J = 9.5, 7.9 Hz, 1H), 4.77 (d, J = 11.2 Hz, 1H), 4.59 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11.2 Hz, 1H), 4.49 (d, J = 11.7 Hz, 1H), 4.44 (d, J = 7.8 Hz, 1H), 4.36−4.33 (m, 2H), 4.25 (dd, J = 11.7, 1.6 Hz, 1H), 4.17−4.12 (m, 2H), 4.09−4.05 (m, 3H), 3.87 (t, J = 9.5 Hz, 1H), 3.80−3.78 (m, 2H), 3.70 (dd, J = 10.2, 3.0 Hz, 1H), 3.60 (dd, J = 8.6, 2.6 Hz, 1H), 3.52 (ddd, J = 9.6, 4.3, 2.1 Hz, 1H), 3.44 (dt, J = 8.1, 2.7 Hz, 1H), 2.13 (m, 3H), 2.06 (s, 3H), 1.92 (s, 3H), 1.72−1.45 (m, 8H), 1.25−1.24 (m, 66H), 1.23 (s, 9H), 1.22 (s, 9H), 1.18 (s, 18H), 1.15 (s, 9H), 1.14 (s, 9H), 1.11 (d, J = 6.5 Hz, 3H), 0.88−0.85 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.8, 177.7, 177.0,

176.9, 176.7, 175.9, 172.4, 170.6, 170.2, 169.7, 138.7, 138.6, 128.35, 128.31, 127.8, 127.7, 127.6, 127.5, 101.1, 100.2, 80.0, 78.5, 74.8, 73.7, 73.5, 73.3, 72.1, 72.0, 71.6, 71.2, 70.9, 69.9, 68.9, 67.9, 67.2, 65.5, 65.2, 61.9, 61.6, 49.8, 39.0, 38.9, 38.83, 38.80, 38.7, 38.6, 36.7, 36.6, 31.9, 29.8, 29.7, 29.69, 29.65, 29.5, 29.4, 29.39, 29.35, 27.3, 27.2, 27.18, 27.13, 27.0, 26.1, 25.6, 24.7, 23.4, 22.7, 21.1, 20.6, 20.5, 15.7, 14.1; HRMS calcd for $C_{112}H_{185}NO_{27}Na$ ([M + Na]$^+$) 1999.3032, found 1999.2950.

General Procedure for the Preparation of iGb3 analogues 35−38. 2-Deoxy-α-D-galactopyranosyl-(1,3)-β-D-galactopyranosyl-(1,4)-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylaminooctadecen-3,4-diol (2-dh-iGb3) (35). A suspension of glycoceramide **31** (46 mg, 0.025 mmol) and $Pd(OH)_2$/C (30 mg, 20 wt %) in MeOH (3 mL) was shaken for 4 h under H_2 atmosphere (50 psi). The catalytic $Pd(OH)_2$/C was filtered off through a celite pad and the filtrate was concentrated. Then the residue was dissolved in anhydrous MeOH (5 mL) and freshly prepared NaOMe (4 mg, 0.07 mmol) was added. The resulting mixture was heated to reflux for 24 h. After the solution was cooled to room temperature, the precipitate was collected by centrifuge. The precipitate was washed with MeOH (2 × 2 mL) and dissolved in pyridine. The insoluble impurities were removed by centrifuge and the clear solution was concentrated to give 2-dh-iGb3 **35** (12 mg, 41%) as white powder. ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.51 (d, J = 8.8 Hz, 1H), 7.54 (s, 1H), 7.32 (d, J = 4.5 Hz, 1H), 6.62 (s, 1H), 6.46−6.44 (m, 2H), 6.41−6.38 (m, 2H), 6.15 (s, 1H), 6.11−6.09 (s, 2H), 5.84 (d, J = 6.7 Hz, 1H), 5.67 (s, 1H), 5.10−5.05 (m 2H), 4.91 (t, J = 5.9 Hz, 2H), 4.86 (d, J = 7.7 Hz, 1H), 4.76 (dd, J = 10.5, 5.7 Hz, 1H), 4.62 (m, 2H), 4.58−4.54 (m, 1H), 4.49 (s, 1H), 4.43−4.40 (m, 6H), 4.36−4.30 (m, 4H), 4.23−4.17 (m, 3H), 4.00−3.97 (m, 1H), 3.80 (br, 1H), 2.51 (td, J = 12.1, 3.1 Hz, 1H), 2.42 (t, J = 7.4 Hz, 2H), 2.23−2.19 (m, 2H), 1.95−1.89 (m, 2H), 1.85−1.77 (m, 2H), 1.73−1.63 (m, 1H), 1.32−1.25 (m, 65H), 0.88−0.85 (m, 6H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.6, 105.7, 105.3, 94.6, 82.1, 77.9, 77.0, 76.7, 76.5, 75.9, 74.7, 72.7, 72.5, 70.7, 70.5, 69.7, 66.2, 65.3, 63.0, 62.1, 62.0, 52.2, 36.9, 34.1, 33.6, 32.2, 30.4, 30.2, 30.1, 30.0, 29.99, 29.97, 29.89, 29.8, 29.67, 29.65, 26.7, 26.4, 23.0, 14.3; HRMS calcd for $C_{62}H_{119}NO_{18}Na$ ([M + Na]$^+$) 1188.8325, found 1188.8257.

3-Deoxy-α-D-galactopyranosyl-(1,3)-β-D-galactopyranosyl-(1,4)-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylaminooctadecen-3,4-diol (3-dh-iGb3) (36). Compound **36** (20 mg, 62%) was obtained from compound **32** (55 mg, 0.028 mmol). ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.45 (d, J = 8.7 Hz, 1H), 7.54 (m, 1H), 7.30 (d, J = 4.9 Hz, 1H), 6.59 (m, 1H), 6.48 (m, 1H), 6.44−6.40 (m, 2H), 6.37−6.36 (m 2H), 6.10 (s, 1H), 5.80 (d, J = 7.0 Hz, 1H), 5.75 (s, 1H), 5.55 (d, J = 2.7 Hz, 1H), 5.14−5.09 (m 1H), 5.04 (d, J = 7.8 Hz, 1H), 4.89−4.87 (m, 2H), 4.76 (dd, J = 10.4, 5.2 Hz, 1H), 4.56−4.52 (m, 3H), 4.43−4.40 (m, 4H), 4.38−4.33 (m, 4H), 4.31−4.25 (m, 2H), 4.23−4.18 (m, 2H), 4.02−4.00 (m, 2H), 3.83−3.81 (m, 1H), 2.43−2.40 (m, 4H), 2.21 (m, 1H), 1.93−1.91 (m, 2H), 1.82−1.78 (m, 2H), 1.68 (m, 1H), 1.31−1.25 (m, 67H), 0.88−0.85 (m, 6H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.4, 105.5, 105.3, 97.0, 82.1, 79.9, 76.7, 76.6, 76.5, 75.9, 74.7, 72.7, 71.9, 70.7, 70.6, 67.1, 66.1, 64.5, 62.8, 62.0, 61.8, 52.2, 36.9, 36.1, 33.6, 32.2, 32.1, 30.4, 30.2, 30.1, 30.0, 29.96, 29.93, 29.85, 29.78, 29.65, 29.63, 26.7, 26.4, 23.0, 14.3; HRMS calcd for $C_{62}H_{119}NO_{18}Na$ ([M + Na]$^+$) 1188.8325, found 1188.8328.

4-Deoxy-α-D-galactopyranosyl-(1,3)-β-D-galactopyranosyl-(1,4)-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylaminooctadecen-3,4-diol (4-dh-iGb3) (37). Compound **37** (16 mg, 69%) was obtained from compound **33** (39 mg, 0.020 mmol). ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.54 (d, J = 8.8 Hz, 1H), 7.60 (s, 1H), 7.37 (d, J = 5.2 Hz, 1H), 6.72 (d, J = 3.9 Hz, 1H), 6.67 (m, 1H), 6.51 (d, J = 6.2 Hz, 1H), 6.44−6.42 (m, 2H), 6.13 (s, 1H), 5.89 (d, J = 7.1 Hz, 1H), 5.84 (s, 1H), 5.60 (d, J = 3.5 Hz, 1H), 5.14−5.09 (m 1H), 4.94 (m, 1H), 4.87 (d, J = 8.0 Hz, 2H), 4.77 (dd, J = 10.6, 5.4 Hz, 1H), 4.57 (br, 2H), 4.52−4.47 (m, 1H), 4.43 (br, 3H), 4.38−4.35 (m, 2H), 4.33−4.29 (m, 1H), 4.23−4.17 (m, 4H), 4.06−3.99 (m, 5H), 3.81−3.79 (m, 1H), 3.59 (d, J = 5.1 Hz, 1H), 2.48−2.44 (m, 1H), 2.42 (t, J = 7.5 Hz, 2H), 2.25−2.19 (m, 1H), 2.05 (ddd, J = 12.2, 12.0, 12.0 Hz, 1H), 1.96−1.90 (m, 2H), 1.84−1.77 (m, 2H), 1.68 (m, 1H), 1.31−1.24 (m, 67H), 0.88−0.84 (m, 6H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.4, 105.5, 105.3, 97.8, 82.1, 80.2, 76.7, 76.5, 75.9, 75.6, 74.7, 72.7, 70.7, 70.5, 68.5, 66.0, 65.5, 62.0, 61.8, 52.2, 37.2, 36.9, 33.6, 32.2, 30.4, 30.2, 30.1, 30.0, 29.96, 29.94, 29.86, 29.78, 29.63, 26.7, 26.4, 23.0, 14.3; HRMS calcd for $C_{62}H_{119}NO_{18}Na$ ([M + Na]$^+$) 1188.8325, found 1188.8330.

6-Deoxy-α-D-galactopyranosyl-(1,3)-β-D-galactopyranosyl-(1,4)-β-D-glucopyranosyl-(1,1)-(2*S*,3*S*,4*R*)-2-hexacosanoylaminooctadecen-3,4-diol (6-dh-iGb3) (38). Compound **38** (23 mg, 50%) was obtained from compound **34** (80 mg, 0.04 mmol). ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.49 (d, J = 8.7 Hz, 1H), 7.26 (d, J = 5.1 Hz, 1H), 6.61 (s, 1H), 6.48 (br, 1H), 6.45 (d, J = 5.2 Hz, 1H), 6.39 (s, 1H), 6.16 (s, 1H), 6.13 (s, 1H), 5.82 (d, J = 7.0 Hz, 1H), 5.57 (d, J = 2.8 Hz, 1H), 5.14−5.11 (m 1H), 5.06 (d, J = 7.9 Hz, 1H), 4.88 (d, J = 7.9 Hz, 2H), 4.77 (dd, J = 10.6, 5.4 Hz, 1H), 4.65−4.62 (m, 1H), 4.57 (s, 1H), 4.52−4.51 (m, 2H), 4.45 (m, 3H), 4.38−4.36 (m, 3H), 4.22 (d, J = 8.7 Hz, 3H), 4.12 (s, 1H), 4.06 (t, J = 5.9 Hz, 1H), 4.01 (t, J = 7.9 Hz, 1H), 3.82−3.80 (m, 1H), 3.61 (s, 1H), 2.43 (t, J = 7.3 Hz, 2H), 2.26−2.20 (m, 1H), 1.95−1.93 (m, 2H), 1.85−1.79 (m, 2H), 1.71−1.68 (m, 1H), 1.58 (d, J = 6.4 Hz, 3H), 1.31−1.27 (m, 68H), 0.89−0.87 (m, 6H); ^{13}C NMR (125 MHz, pyridine-d_5) δ 173.8, 105.8, 105.6, 98.0, 82.4, 80.4, 77.0, 76.9, 76.8, 76.2, 75.0, 73.8, 73.0, 72.0, 71.0, 70.9, 70.4, 68.1, 66.3, 62.4, 62.2, 52.5, 37.3, 33.9, 32.5, 30.7, 30.5, 30.4, 30.38, 30.31, 30.28, 30.21, 30.1, 30.0, 29.97, 27.0, 26.7, 23.3, 17.6, 14.6; HRMS calcd for $C_{62}H_{119}NO_{18}Na$ ([M + Na]$^+$) 1188.8325, found 1188.8322.

Bioassay Experiment. Stimulatory activities of glycolipids were assayed by a murine iNKT stimulation system as described,[8] using bone marrow-derived mouse DC as APC and a murine iNKT hybridoma, DN32D3, as responder cells. DN32D3 is a NKT cell hybridoma that secrets IL2 upon recognition of glycolipids presented by dendritic cells. iGb3 and its analogues (**35**−**38**) were dissolved at 1 mg/mL in DMSO and stored at −20 °C. In this experiment, they were further diluted in cell culture medium at indicated concentrations and pulsed to 100 000 mouse (C57BL6) bone marrow derived dendritic cells for 8 h, after which the dendritic cells were washed with culture medium and mixed with 50 000 DN32.D3 hybridoma cells. Then the resulted mixture was co-cultured for 24 h, and the released IL2 was measured by CTLL assay.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds **1**−**3**, **8**, **11**−**18**, **22**−**25**, **27**−**38** and ^{1}H−^{1}H COSY of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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