Solution and Solid-Support Synthesis of a Potential Leishmaniasis Carbohydrate Vaccine

Michael C. Hewitt and Peter H. Seeberger*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

seeberg@mit.edu

Received January 12, 2001

The synthesis of a potential carbohydrate vaccine for the parasitic disease leishmaniasis is described. New solution- and solid-phase synthetic strategies were explored for the assembly of a unique tetrasaccharide antigen found on the *Leishmania* lipophosphoglycan. An initial solution-phase synthesis relied on thioglycosides as building blocks and the establishment of the central disaccharide from lactal via an oxidation−reduction sequence. A second approach was completed both in solution and on solid support. The solid-phase synthesis relied on assembly from monosaccharide units and was used to evaluate different glycosylating agents in the efficient installation of the galactose β-(1→4) mannoside. Glycosyl phosphates proved most successful in this endeavor. This first solid-phase synthesis of the *Leishmania* cap provided rapid access to the tetrasaccharide in 18% overall yield while requiring only a single purification step. The synthetic cap tetrasaccharide was conjugated to the immunostimulator Pam_3Cys to create fully synthetic carbohydrate vaccine **1** and to the carrier protein KLH to form semisynthetic vaccine **2**. Currently, both constructs have entered initial immunological experiments in mice targeted at the development of a vaccine against the parasitic disease leishmaniasis.

Introduction

Leishmaniasis is a tropical disease that afflicts over 12 million people worldwide, with 1.5−2 million new cases estimated to occur each year.[1] Three different forms of the disease, spread by the bite of infected sandflies, arise in humans. Visceral leishmaniasis, also known as *kala azar*, causes swelling of the spleen and liver, and is often lethal when left untreated. The most common form of the disease, cutaneous leishmaniasis, results in debilitating skin lesions, while mucocutaneous leishmaniasis causes lesions in the mucous membranes, leading to facial disfigurement. Although leishmaniasis is most prevalent in tropical settings, the disease has recently been diagnosed in overseas travelers and U.S. Gulf War veterans,[2] and has emerged as an opportunistic infection of HIV patients.[3] It is feared that the disease is currently becoming endemic in the U.S.[4]

Leishmaniasis has been extensively studied in murine models,[5] but low parasite levels in humans render the diagnosis of cutaneous and mucocutaneous leishmaniasis difficult. Even after diagnosis, the mechanism of infection poses a serious challenge for therapeutic approaches. The parasite resides within macrophages, the very part of the immune response that is designed to destroy it. Treatment with pentavalent antimony medications such as Pentostam and Glucantime is hampered by lengthy treatment protocols (leading to low compliance) and toxic side effects.[6]

A potent vaccine that would facilitate destruction of the parasite upon transfer from the sandfly into the human host would be ideal. To date, several different vaccine designs have been explored, each focusing on a different part of the parasite in the hope of inducing a specific immune response. Killed or attenuated parasites did not result in valid vaccines, and leishmanial promastigote surface protease gp63 vaccines are in various phases of testing. Thus, no generally applicable vaccine for leishmaniasis has yet emerged.[7]

Here we describe the preparation of fully synthetic and semisynthetic immunogens that target leishmaniasis based on the unique tetrasaccharide cap of the parasite's cell-surface lipophosphoglycan. New synthetic strategies for the solution- and solid-phase synthesis of this carbohydrate unit containing the rare galactose β-(1→4) mannoside core are reported and different glycosylating agents were explored. Two immunogens were prepared by joining the tetrasaccharide antigen with the immunostimulator tripalmitoyl-*S*-glycerylcysteine and with keyhole limpet hemocyanin (KLH). Both the fully synthetic and the semisynthetic immunogens are currently undergoing immunological evaluation in mice.

Vaccine Design Considerations

In principle, any unique feature the parasite exposes on its cell surface may be used for the development of a leishmaniasis vaccine. Lipophosphoglycans (LPGs, Figure 1),[8] which are ubiquitous on the cell surface of *Leishmania* parasites, are composed of a glycosylphosphatidylinositol (GPI) anchor, a repeating phosphorylated

(1) For a review, see Herwaldt, B. L. *Lancet* **1999**, *354*, 1191.
(2) Magill, A. J.; Grogl, M.; Gasser, R. A.; Sun, W.; Oster, C. N. *N. Engl. J. Med.* **1993**, *328*, 1383.
(3) Alvar, J.; Canavate, C.; Gutierrez-Solar, B.; et al. *Clin. Microbiol. Rev.* **1997**, *10*, 298
(4) Enserink, M. *Science* **2000**, 290, 1881.
(5) Reed, S. G.; Scott, P. *Curr. Opin. Immunol.* **1993** *5*, 524.
(6) Berman, J. D. *Clin. Infect. Dis.* **1997**, *24*, 684
(7) Modabber, F. *Ann. Trop. Med. Parasitol.* **1995**, *89* (suppl 1), 83.
(8) For a review, see Turco, S. J.; Descoteaux, A. *Annu. Rev. Microbiol.* **1992**, *46*, 65.

10.1021/jo015521z CCC: $20.00
Published on Web 05/19/2001

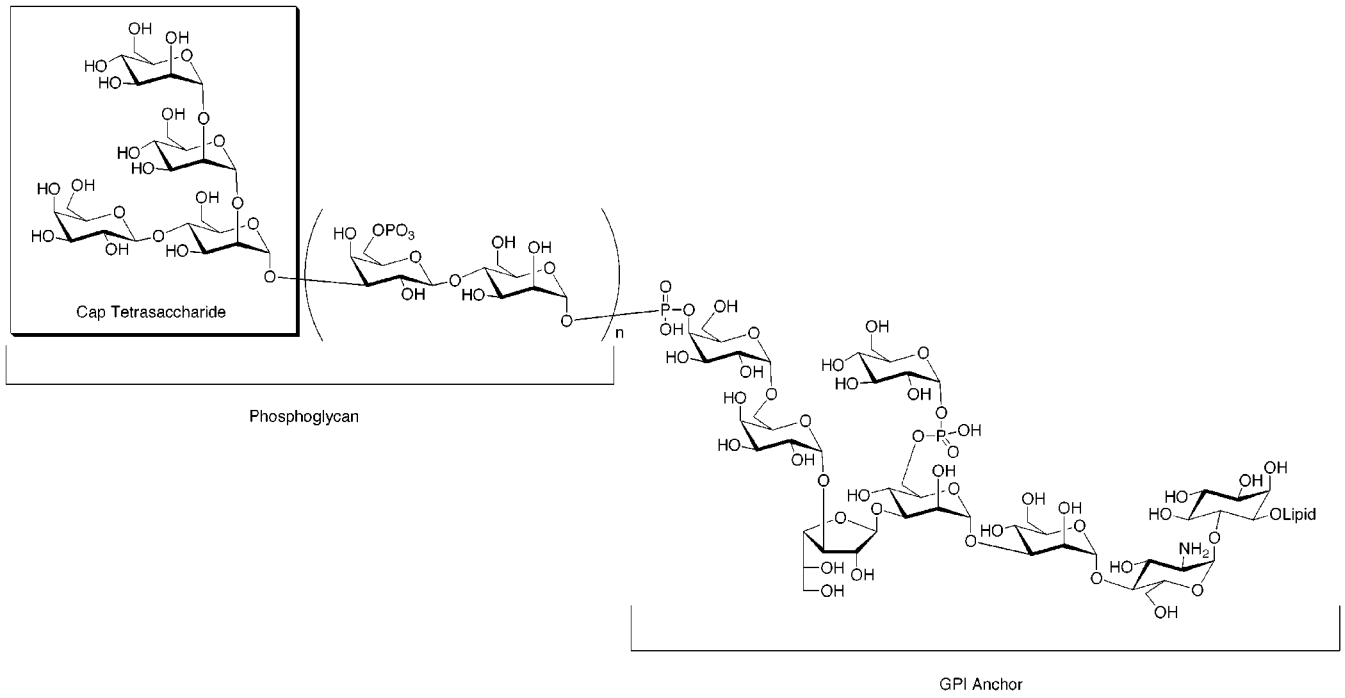


Figure 1. Leishmania lipophosphoglycan.

disaccharide, and different cap oligosaccharides. The LPGs have been examined as vaccine candidates since the phosphoglycan portion of LPG was shown to be a disease-promoting antigen,[9] but the size (MW ca. 12000) and heterogeneity of the diverse LPGs preclude their use. The presence of unique oligosaccharide structures on the surface of the parasite attracted our attention in contemplating the use of synthetic carbohydrate chemistry to create vaccines against tropical diseases. In particular, a unique tetrasaccharide containing the unusual galactose β-(1→4) mannosidic linkage piqued our interest from a synthetic standpoint. Thus, we began to investigate the feasibility of developing a structurally well-defined leishmaniasis carbohydrate vaccine, based on this cap tetrasaccharide.

Different oligosaccharide vaccine constructs have recently been investigated in the context of anticancer vaccines based upon tumor-associated carbohydrate antigens.[10] Semisynthetic constructs were readily prepared by covalent attachment of the synthetic oligosaccharide antigen to a carrier protein such as KLH or BSA (bovine serum albumin).[11] Several semisynthetic constructs have been tested in in vivo experiments and have entered various phases of clinical trials against a host of cancers.[12] The initial clinical results are quite promising and validate this approach.

The structural ambiguity of the carbohydrate−protein conjugates due to the ill-defined nature of the carrier proteins prompted several groups to explore fully synthetic carbohydrate vaccines that do not require additional adjuvants. The lipopeptide tripalmitoyl-*S*-glycerylcysteine (Pam_3Cys) can be readily prepared,[13] and serves both as a carrier and adjuvant.[14] Upon conjugation to an antigen the result is a homogeneous, low molecular weight construct that is readily taken up by antigen-presenting cells. The stability of these synthetic vaccines to heat, light, and different solvents renders them particularly attractive for use in tropical settings.[15] A selective immune response against glycoproteins expressing a carbohydrate T_N antigen (GalNAcα1 → O−Ser/Thr)[16] has been reported using a fully synthetic Pam_3Cys-T_N vaccine.[17]

Given the success of semisynthetic and synthetic carbohydrate vaccines against different diseases, we designed two potential *Leishmania* vaccine constructs (Figure 2). In one case the synthetic cap tetrasaccharide was to be connected to Pam_3Cys to fashion fully synthetic vaccine **1**, while a KLH-conjugate would provide semisynthetic vaccine **2**.

Solution-Phase Synthesis of the *Leishmania* Cap Tetrasaccharide. Different sections of the *Leishmania* LPG have served as targets for synthetic studies, including the GPI heptasaccharyl *myo*-inositol.[18] Synthetic phosphorylated oligosaccharides[19] of the *Leishmania Donovani* LPG were analyzed as substrates for *Leish-*

(9) Mitchell, G. F.; Handman, E. *Parasite Immun.* **1986**, *8*, 255.
(10) Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **2000**, *39*, 836.
(11) Francis, M. J. In *Vaccines: Recent Trends and Progress*; Gregoriadis, G., Allison, A. C., Poste, G., Eds.; Plenum Press: New York, 1991.
(12) (a) Ragupathi, G.; Slovin, S. F.; Adluri, S.; Sames, D.; Kim, I. J.; Kim, H. M.; Spassova, M.; Bornmann, W. G.; Lloyd, K. O.; Scher, H. I.; Livingston, P. O.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 563. (b) Ragupathi, G.; Park, T. K.; Zhang, S.; Kim, I. J.; Graber, L.; Adluri, S.; Lloyd, K. O.; Danishefsky, S. J.; Livingston, P. O. *Angew. Chem., Int. Ed.* **1997**, *36*, 125. (c) Slovin, S. F.; Ragupathi, G.; Adluri, S.; Ungers, G.; Terry, K.; Kim, S.; Spassova, M.; Bornmann, W. G.; Fazzari, M.; Dantis, L.; Olkiewicz, K.; Lloyd, K. O.; Livingston, P. O.; Danishefsky, S. J.; Scher, H. I. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5710.
(13) Wiesmuller, H.-K.; Bessler, W. G.; Jung, G. *Hoppe-Seylers Z. Physiol. Chem.* **1983**, *364*, 593.
(14) Reichel, F.; Ashton, P. R.; Boons, G.-J. *Chem. Commun.* **1997**, 2087;
(15) Muller, C. P.; Jung, G. *Lancet* **1988**, 1424
(16) (a) Springer, G. F. *Science* **1984**, *224*, 1198. (b) Itzkowitz, S. H.; Yuan, M.; Montgomery, C. K.; Kjeldsen, T.; Takahashi, H. K.; Bigbee, W. L.; Kim, Y. S. *Cancer Res.* **1989**, *49*, 197.
(17) Toyokuni, T.; Dean, B.; Cai, S.; Boivin, D.; Hakomori, S.-I.; Singhal, A. K. *J. Am. Chem. Soc.* **1994**, *116*, 395.
(18) Ruda, K.; Lindberg, J.; Garegg, P. J.; Oscarson, S.; Konradsson, P. *J. Am. Chem. Soc.* **2000**, *122*, 11067.

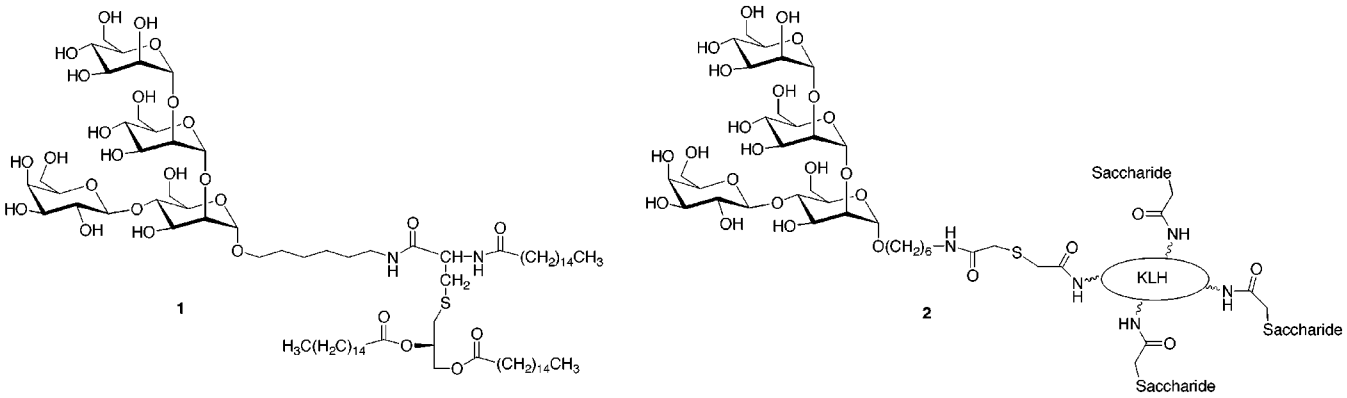
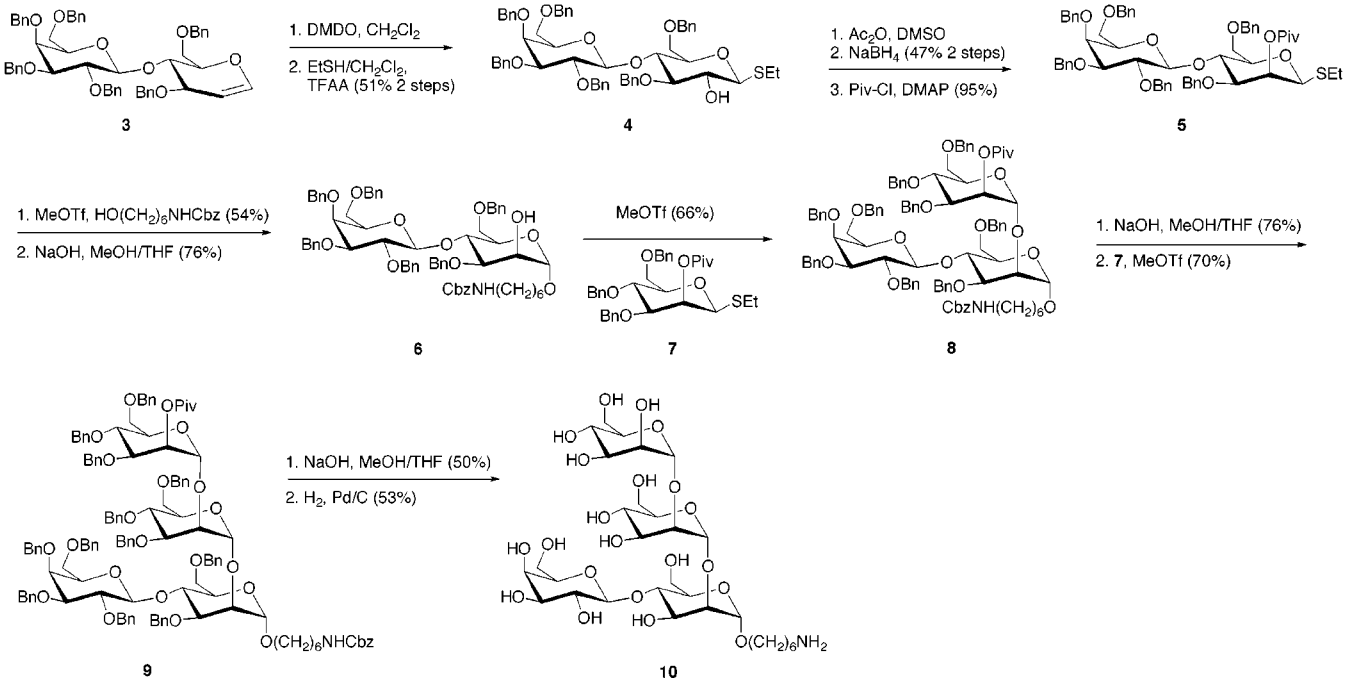


Figure 2. Synthetic and semisynthetic leishmaniasis vaccine constructs.

Scheme 1. Solution-Phase Synthesis of the Leishmania Cap Tetrasaccharide



mania transferase.[20] The cap tetrasaccharide itself has also been the subject of a previous synthesis.[21] Fraser-Reid et al. explored the utility of *n*-pentenyl glycosides[22] as building blocks to create a fully protected cap structure in a both a linear and convergent fashion. The modest yield (35%) for the final coupling between a trisaccharide acceptor and monosaccharide donor as well as several protecting group manipulations during assembly left room for improvement. The tetrasaccharide was not liberated from the protecting groups but rather served as a formidable synthetic challenge to explore new methodology. A radiolabeled version of the cap tetrasaccharide was also recently prepared by Upreti et al.[23]

Hexabenzyl lactal **3**[24] served as starting point for the solution synthesis (Scheme 1). Epoxidation of the glycal double bond under the agency of 2,2′-dimethyldioxirane and reaction with ethanethiol in the presence of trace amounts of acid resulted in thioethyl lactose **4**.[25] Further conversion by a two step oxidation−reduction process[26] to invert the stereochemistry of the C2 position furnished the desired thioethyl galactose-β-(1→4)-mannoside **5** after pivaloylation. Thioethyl glycoside **5** served as glycosyl donor in the union with *N*-Cbz protected aminohexanol[27] employing methyl triflate (MeOTf) activation to yield 54% of the desired α-glycoside and permanently installed the protected amino tether. Removal of the C2-pivaloyl-protected alcohol with sodium hydroxide provided disaccharide acceptor **6.** Coupling of 2-pivaloyl mannosyl thiodonor **7**[28] with disaccharide **6** provided protected trisaccharide **8**. Further elongation by repetition of the deprotection/coupling steps furnished the fully protected

(19) (a) Nikolaev, V. A.; Rutherford, T. J.; Ferguson, M. A. J.; Brimacombe, J. S. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1559. (b) Nikolaev, A. V.; Rutherford, T. J.; Ferguson, M. A. J.; Brimacombe, J. S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1977. (c) Nikolaev, A. V.; Chudek, J. A.; Ferguson, M. A. J. *Carbohydr. Res.* **1995**, *272*, 179.

(20) Brown, G. M.; Millar, A. R.; Masterson, C.; Brimacombe, J. S.; Nikolaev, A. V.; Ferguson, M. A. J. *Eur. J. Biochem.* **1996**, *242*, 410.

(21) Arasappan, A.; Fraser-Reid, B. *J. Org. Chem.* **1996**, *61*, 2401.

(22) Madsen, R.; Fraser-Reid, B. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neil, R. A. Eds.; Harwood Academic Publishers: Switzerland, 1995; Chapter 4.

(23) Upreti, M.; Ruhela, D.; Vishwakarma, R. A. *Tetrahedron* **2000**, 6577.

(24) Kinzy, W.; Schmidt, R. R. *Carbohydr. Res.* **1987**, *164*, 265.

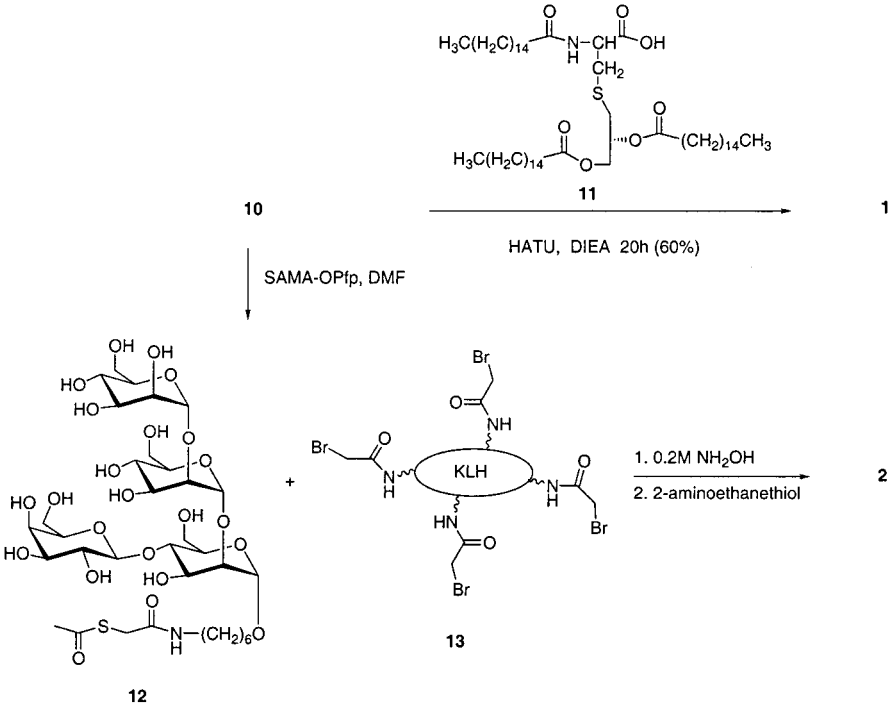
(25) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064.

(26) Albright, J. D.; Goldman, L. *J. Am. Chem. Soc.* **1967**, *89*, 2416.

(27) Muller, D.; Vic, G.; Critchley, P.; Crout, D. H. G.; Lea, N.; Roberts, L.; Lord, J. M. *J. Chem. Soc. Perkin Trans. 1* **1998**, 2287.

(28) Seeberger, P. H.; Eckhardt, M.; Gutteridge, C. E.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1997**, *119*, 10064

Scheme 2. Synthesis of Synthetic and Semisynthetic Carbohydrate Vaccine Constructs

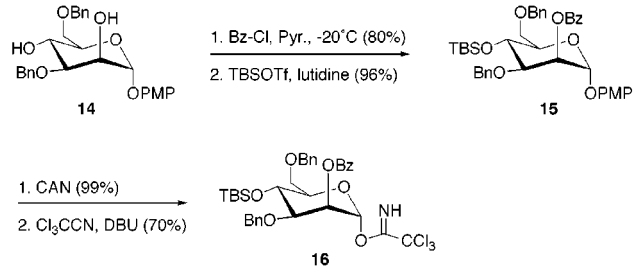


cap tetrasaccharide **9** equipped with an aminohexyl spacer on the reducing terminus. No evidence for formation of the undesired β-isomers were detectable by ^{1}H NMR for formation of **8** and **9**. Global deprotection of **9** was achieved via a two-step protocol. Saponification of the pivaloyl ester was followed by removal of the benzyl ethers and the carbamate via palladium-catalyzed hydrogenation to yield tetrasaccharide **10**.

With the linker-equipped *Leishmania* cap tetrasaccharide in hand, we embarked on the preparation of the synthetic and semisynthetic immunogens. The primary amine served as attachment site in the union with the immunostimulator Pam_3Cys using the HATU/HOAt activation method[29] to fashion the amide linkage. The coupling between the lipophilic amino acid and hydrophilic carbohydrate furnished the desired vaccine construct **1** in 60% yield. Purification of **1** was best accomplished by silica column chromatography before characterization by high-resolution mass spectrometry and ^{1}H NMR unequivocally confirmed the structure of the vaccine. It is worth pointing out that concentrated solutions of **1** rapidly form micelles and complicate analysis of **1** significantly.

In addition to the fully synthetic immunogen **1**, and in light of the success achieved with carbohydrate–protein constructs as antitumor agents (vide supra), we focused next on the preparation of a semisynthetic vaccine **2**. Tetrasaccharide amine **10** was condensed with *S*-acetylmercaptoacetate pentafluorophenyl ester to afford **12** in 68% yield (Scheme 2). Similarly, KLH was condensed with *N*-succidimidylbromoacetate to provide bromoacetate-modified protein **13**. Union of **12** and **13** in the presence of NH_2OH was followed by capping of any remaining bromoacetates with 2-aminoethanethiol to furnish semisynthetic vaccine **2**. The conjugation ratio of cap tetrasaccharide to each KLH molecule was 55:1 as determined by carbohydrate and protein analysis. Both the synthetic vaccine **1** and semisynthetic sugar–protein conjugate **2** are currently undergoing initial immunological evaluation in Balb-C mice.

Scheme 3

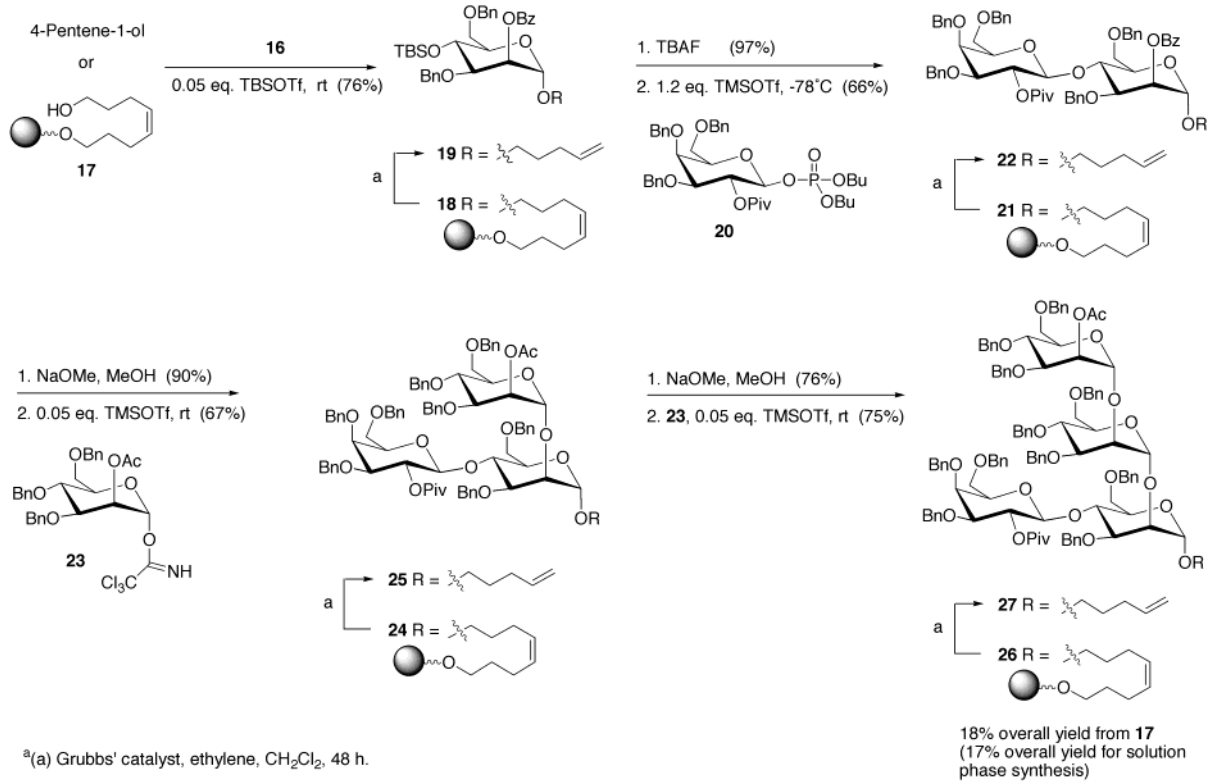


Solid-Phase Synthesis of the *Leishmania* Cap Tetrasaccharide. In addition to establishing a reliable new route for the synthesis of the *Leishmania* cap tetrasaccharide, we intended to develop novel synthetic strategies that would allow us to take advantage of the solid-phase paradigm. Advantages offered by the synthesis of carbohydrates on solid support are improved yields due to the use of excess reagents to drive reactions to completion (3 equiv of donor were used for each glycosylation) and a single purification step at the end of the synthesis rather than chromatographic purification after each transformation in a multistep synthesis. While convenient with respect to the ease of procurement of starting glycals, our initial synthetic approach included several steps that are difficult to carry out on solid support and proceeded in relatively low yields. The oxidation–reduction procedure for the conversion of a glucoside into a mannoside had previously been successfully accomplished even on solid support but resulted in less than quantitative yields.[30] It was this difficult transformation that rendered a synthesis from monosaccharides in a stepwise fashion attractive.

(29) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.

(30) Seeberger, P. H.; Danishefsky, S. J. Unpublished results.

Scheme 4. Solution- and Solid-Phase Synthesis of the *Leishmania* Tetrasaccharide Using Monosaccharide Building Blocks[a]



[a](a) Grubbs' catalyst, ethylene, CH_2Cl_2, 48 h.

Mindful of the fact that a successful solid-phase approach would have to produce the tetrasaccharide in a form that would allow for the attachment of a handle on its reducing end for the adornment of different immunostimulants, the installation of a pentenyl glycoside was very attractive.[31] Recently, we introduced a new linker concept for solid-phase oligosaccharide synthesis based on an octenediol linker, which after olefin cross-metathesis under an atmosphere of ethylene reveals a terminal pentenyl glycoside.[32] The assembly of the cap tetrasaccharide from monosaccharides was accomplished using an identical reaction path both in solution and on the solid support (Scheme 4).

Preparation of the differentially protected mannose core building block **16** commenced with known mannose diol **14** (Scheme 3).[33] Regioselective benzoylation of the axial C2 hydroxyl group was followed by masking of the less reactive C4-hydroxyl as a TBS-ether to furnish fully differentiated **15**. The temporary anomeric *p*-methoxyphenyl protection of **15** was oxidatively removed, and the resulting hemiacetal of mannose was converted into trichloroacetimidate **16** by reaction with trichloroacetonitrile and DBU.[34]

With differentially protected core mannose building block **16** at our disposal, coupling with pentenyl alcohol fashioned *n*-pentenyl glycoside **19** upon activation of the trichloroacetimidate with catalytic amounts of TBSOTf in 1 h at room temperature (Scheme 4). Selective removal of the C4 silyl ether by treatment with TBAF was followed by glycosylation of the exposed C4 hydroxyl group with glycosyl phosphate donor **20**. Galactosyl phosphate **20** was readily prepared in gram quantities from tribenzyl galactal employing a straightforward and high-yielding one-pot procedure.[35] As expected, the use of glycosyl phosphate donors facilitated the glycosylation of the sterically encumbered C4 hydroxyl moiety without complications and completely stereoselectively by virtue of the C2 participating pivaloyl group.[38] Selective removal of the C2 benzoate protection revealed the axial acceptor site for the coupling with trichloroacetimidate mannosyl donor **23** to provide trisaccharide **25**. Removal of the axial acetate and renewed elongation by coupling with **23** furnished cap tetrasaccharide pentenyl glycoside **26**. This stepwise tetrasaccharide assembly proceeded without problem but required the purification of intermediates at seven steps throughout the synthesis.

To further simplify the preparation of the cap tetrasaccharide and to maximize the overall yields, the assembly of the target molecule on solid support was explored. Following the same sequence of reactions as for the solution-phase synthesis (Scheme 4), octenediol linker functionalized Merrifield resin **17** served as the starting point in place of *n*-pentenyl alcohol. At each step of the synthesis a small portion of the resin was set aside and the reaction products were cleaved by olefin cross-metathesis[36] using Grubbs catalyst under an atmosphere of ethylene. Thus, the saccharide products were obtained in the form of the terminal *n*-pentenyl glycosides[37] and compared to the samples obtained by solution-phase

(31) Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958.
(32) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811.
(33) Mori, M.; Ito, Y.,; Ogawa, T. *Carbohydr. Res.* **1989**, *192*, 131.
(34) (a) Rademann, J.; Schmidt, R. R. *Tetrahedron Lett.* **1996**, *37*, 3989, (b) Rademann, J.; Schmidt, R. R. *J. Org. Chem.* **1997**, *62*, 3989. (c) Heckel, A.; Mross, E.; Jung, K.-H.; Rademann, J.; Schmidt, R. R. *Synlett* **1998**, 171, (d) Rademann, J.; Geyer, A.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **1998**, *37*, 1241. (e) Hunt, J. A.; Roush, W. R. *J. Am. Chem. Soc.* **1996**, *118*, 9998. (f) Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1996**, *37*, 5007. (g) Adinolfi, M.; Barone, G.; De Napoli, L.; Iadonisi, A.; Piccialli, G. *Tetrahedron Lett.* **1998**, *39*, 1953.
(35) Plante, O. J.; Andrade, R. B.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 211.

chemistry. Simple washing of the resin was used to remove excess reagents. Cleavage of support-bound tetrasaccharide **25** yielded pure tetrasaccharide *n*-pentenyl glycoside **27** after a single silica column in 18% overall yield from **17**. The solid-phase assembly took 4 days and was significantly faster than the solution-phase synthesis that required at least two weeks.

This solid-phase synthesis demonstrated that branched oligosaccharides of biological interest are accessible in good overall yields using glycosyl trichloroacetimidate and glycosyl phosphate donors in concert. The terminal *n*-pentenyl glycoside could be further elaborated into an aldehyde or carboxylic acid that would allow for attachment to immunostimulators such as proteins or lipopeptides.

Conclusions

In summary, we have introduced two new synthetic routes for the preparation of the parasite-specific *Leishmania* cap tetrasaccharide. The first solution-phase synthesis relied on thioglycosides as building blocks and the establishment of the central disaccharide from lactal via an oxidation−reduction sequence. The synthetic cap tetrasaccharide was conjugated to the immunostimulator Pam_3Cys to create a fully synthetic carbohydrate vaccine and to the carrier protein KLH to form a semisynthetic vaccine. Currently, both vaccine constructs have entered initial immunological experiments in mice targeted at the development of a vaccine against the parasitic disease leishmaniasis. The results of the immunological evaluations will be reported in due course. The second synthetic scheme built on assembly of the target structure from monosaccharides and was readily executed both in solution and on the solid support. It was demonstrated that glycosyl trichloroacetimidates and glycosyl phosphates can be used together to create branched oligosaccharides of biological significance. This first solid-phase synthesis of the *Leishmania* cap provides rapid access to synthetic material while requiring only a single purification step.

Experimental Section

All commercial materials were used without further purification unless otherwise noted. 10x phosphate-buffered saline (PBS) was purchased from Boehringer Mannheim and diluted to the desired concentration; Pd-10 columns (Sephadex G-25) were purchased from Pharmacia. Dichloromethane (CH_2Cl_2) was distilled from calcium hydride under N_2. THF was distilled from sodium/benzophenone under N_2. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Silicycle silica (230−400 mesh). ^{1}H NMR spectra were obtained on either a Varian VXR-300 (300 MHz) or VXR-500 (500 MHz) and are reported in parts per million (δ) relative to chloroform (7.27 ppm). Coupling constants (J) are reported in hertz. ^{13}C NMR spectra were recorded on a VXR-500 (125 MHz) and are reported in δ relative to $CDCl_3$ (77.0 ppm) as an internal reference.

Ethyl 2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-thio-*β*-D-glucopyranoside 4. Hexabenzyl lactal **3**[24] (256 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (600 μL) and cooled to 0 °C. Dimethyldioxirane (15.0 mL in acetone, 0.87 mmol) was added via cannula, and the solution was stirred for 10 min at 0 °C. The solvent was removed in vacuo. After drying in vacuo for 30 min, the epoxide was dissolved in CH_2Cl_2 (714 μL), and EtSH (714 μL) was added. The mixture was cooled to −78 °C, and triflouroacetic anhydride (5 μL) was added dropwise. The reaction mixture was allowed to warm to room temperature over 16 h. The solvent was removed in a stream of N_2. Purification by flash silica column chromatography (10 → 30% EtOAc/hexanes) afforded **4** (143 mg, 51%) as a white solid. $[\alpha]^{24}_D$: −3.92° (*c* 1.0, CH_2Cl_2); IR (thin film) 3447, 3062, 3029, 2867, 1498 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.32−7.11 (m, 30H), 5.04 (d, J = 11.1 Hz, 1H), 4.92 (d, J = 11.5 Hz, 1H), 4.76−4.65 (m, 5H), 4.49−4.20 (m, 8H), 3.95−3.87 (m, 2H), 3.78−3.66 (m, 3H), 3.54−3.32 (m, 7H), 2.65 (q, J = 4.1 Hz, 2H), 2.48 (s, 1H), 1.24 (t, J = 4.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 138.7, 138.6, 138.4, 138.1, 138.0, 137.6, 128.1, 128.1, 128.0, 127.9, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 102.6, 85.3, 83.4, 82.2, 79.7, 79.5, 75.8, 75.1, 74.7, 74.5, 73.4, 73.3, 72.9, 72.4, 72.1, 68.1, 68.0, 24.0, 15.3; FAB MS m/z ($M + Na^+$) calcd 949.3956, found 949.3952.

Ethyl 2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-pivaloyl-thio-*β*-D-mannopyranoside 5. Thioethyl β-D-glucoside **4** (1.42 g, 1.48 mmol) was treated with DMSO/Ac_2O (10 mL/5 mL) for 72 h at room temperature. The solvents were removed in vacuo. The residue was diluted with EtOAc (50 mL) and washed with sat. $NaHCO_3$ (2 × 100 mL), H_2O (1 × 100 mL), and brine (1 × 100 mL). The crude residue was dried with Na_2SO_4 and concentrated to dryness. After azeotroping (3 × 20 mL toluene) and drying in vacuo for 1 h, the orange oil was dissolved in CH_2Cl_2/MeOH (7 mL/7 mL) and cooled to 0 °C. $NaBH_4$ (265 mg, 7.4 mmol) was added, and the reaction mixture was allowed to warm to room temperature over 2 h. The reaction was quenched with $NaHCO_3$ (30 mL) and diluted with EtOAc (30 mL), and NH_4Cl (30 mL) was added to dissolve solids before the phases were separated. Following extraction of the aqueous phase with EtOAc (3 × 30 mL), the combined extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated. The crude material was purified by flash silica column chromatography (20 → 30% EtOAc/hexanes), affording ethyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-thio-β-D-mannopyranoside (660 mg, 47%) as a clear oil. $[\alpha]^{24}_D$: − 11.5° (*c* 1.0, CH_2Cl_2); IR (thin film) 3447, 3029, 2922, 2866, 1496 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.41−7.27 (m, 30H), 5.00 (d, J = 11.6 Hz, 1H), 4.86 (d, J = 7.0 Hz, 1H), 4.84 (d, J = 6.7 Hz, 1H), 4.77−4.69 (m, 4H), 4.62−4.60 (m, 2H), 4.52 (d, J = 7.9 Hz, 1H), 4.51 (d, J = 11.9 Hz, 1H), 4.43 (d, J = 11.9 Hz, 1H), 4.41−4.07 (m, 2H), 3.95 (d, J = 2.7 Hz, 1H), 3.89−3.86 (m, 1H), 3.80−3.76 (m, 2H), 3.65−3.62 (m, 1H), 3.55 (dd, J = 3.4, 8.8 Hz, 1H), 3.52−3.62 (m, 4H), 2.79−2.74 (m, 2H), 2.62 (d, J = 1.5 Hz, 1H), 1.34 (t, J = 7.3 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 138.8, 138.5, 138.3, 138.2, 138.1, 137.8, 128.3, 128.2, 128.1, 128.1, 128.0, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.2, 127.2, 103.0, 83.5, 82.5, 80.6, 79.9, 79.8, 75.1, 74.5, 74.4, 73.4, 73.4, 73.1, 73.0, 72.5, 72.4, 70.3, 69.0, 25.6, 15.3; FAB MS m/z ($M + Na^+$) calcd 949.3956, found 949.3996.

2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-thio-*β*-D-mannopyranoside (883 mg, 0.92 mmol) was dissolved in CH_2Cl_2 (10 mL), and DMAP (447 mg, 3.66 mmol) was added. After stirring for 30 min, pivaloyl chloride (225 μL, 1.83 mmol) was added. The reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with sat. $NaHCO_3$ (30 mL), and the aqueous phase was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by flash silica column chromatography (20% EtOAc/hexanes) afforded **5** (900 mg, 95%) as a clear oil. $[\alpha]^{24}_D$: − 21.5° (*c* 1.0, CH_2Cl_2); IR (thin film) 2966, 2925, 2867, 1733, 1496 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.40−7.24 (m, 25H), 5.68 (d, J = 3.36 Hz, 1H), 5.01 (d, J = 11.6 Hz, 1H), 4.87 (d, J = 11.0 Hz, 1H), 4.79−4.62 (m, 8H), 4.54 (d, J = 11.9

(36) (a) Schwab, P.; Grubbs, R. H.; Ziller, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 100. (b) Grubbs, R. H.; Miller, S. J.; Fu, G. C. *Acc. Chem. Res.* **1995**, *28*, 446. (c) Schmalz, H. G. *Angew. Chem., Int. Ed. Engl.* **1996**, *34*, 1833.

(37) Buskas, T.; Soderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958.

Hz, 1H), 4.34 (s, 2H), 4.16−4.12 (m, 1H), 3.96−3.79 (m, 4H), 3.68 (dd, J = 3.4, 9.2 Hz, 1H), 3.61−3.58 (m, 2H), 3.51 (dd, J = 2.8, 9.8 Hz, 1H), 3.38−3.34 (m, 2H), 2.78 (q, J = 7.2 Hz, 2H), 1.36 (t, J = 7.6 Hz, 3H), 1.25 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.3, 138.7, 138.5, 138.4, 138.4, 138.2, 137.6, 128.2, 128.0, 127.9, 127.7, 127.5, 127.3, 127.3, 127.2, 127.2, 127.1, 126.8, 102.5, 82.6, 82.3, 79.9, 79.7, 79.3, 75.0, 74.5, 73.8, 73.3, 73.2, 72.8, 72.7, 72.4, 71.4, 69.7, 68.9, 68.0, 39.0, 27.2, 25.7, 15.1; FAB MS m/z (M^+ + H) calcd 1010.4639, found 1010.4671.

6-(Benzyloxycarbonylamino)hexyl 2,3,4,6-Tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside 6. A mixture of **5** (542 mg, 0.52 mmol) and Cbz-protected aminohexanol (286 mg, 0.78 mmol) were azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. CH_2Cl_2 (11 mL), freshly dried 4 Å molecular sieves (500 mg), and di-*tert*-butylpyridine (174 μL, 2.08 mmol) were added, and the mixture was allowed to stir for 30 min at room temperature. After cooling to 0 °C, methyl triflate (234 μL, 2.08 mmol) was added dropwise. After stirring at 0 °C for 20 h, the mixture was warmed to room temperature, and triethylamine (1 mL) was added. The reaction mixture was diluted with EtOAc (20 mL), filtered, and washed with sat. $NaHCO_3$ (2 × 30 mL) and brine (1 × 30 mL). Following drying (Na_2SO_4) and concentration the crude product was purified by flash silica column chromatography (20 → 30% EtOAc/hexanes) to afford 6-(Benzyloxycarbonylamino)hexyl 2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranoside (245 mg, 54%) as a clear oil. $[\alpha]^{24}_D$: −7.6° (*c* 2.40, CH_2Cl_2); IR (thin film) 2824, 1727, 1454, 1243, 1097 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.17 (m, 35H), 5.32 (dd, J = 1.8, 3.1 Hz, 1H), 5.01 (s, 2H), 4.92 (d, J = 11.6 Hz, 1H), 4.82−4.78 (m, 2H), 7.72 (s, 2H), 4.70−4.66 (m, 3H), 4.62 (d, J = 11.6 Hz, 2H), 4.56 (d, J = 11.3 Hz, 1H), 4.53 (d, J = 6.7 Hz, 1H), 4.42 (d, J = 12.2 Hz, 1H), 4.26 (d, J = 0.9 Hz, 2H), 4.15−4.13 (m, 1H), 3.91 (dd, J = 3.4, 9.2 Hz, 1H), 3.87 (d, J = 2.7 Hz, 1H), 3.81 (d, J = 8.9 Hz, 2H), 3.75−3.67 (m, 3H), 3.53−3.49 (m, 1H), 3.42−3.39 (m, 2H), 3.26 (q, J = 5.2 Hz, 2H), 3.20−3.16 (m, 2H), 1.60−1.34 (m, 11H), 1.13 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.5, 156.2, 138.9, 138.8, 138.7, 138.5, 138.3, 137.4, 136.5, 128.4, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.1, 127.1, 127.0, 126.9, 126.7, 102.7, 97.4, 82.6, 79.9, 76.2, 74.9, 74.4, 74.3, 73.2, 73.1, 72.8, 72.7, 72.6, 72.6, 72.4, 72.3, 71.3, 71.0, 68.8, 68.7, 68.0, 67.6, 66.4, 58.4, 40.9, 38.8, 29.8, 29.4, 29.1, 27.0, 27.0, 26.9, 26.4, 26.4, 25.7, 25.7; FAB MS m/z (M^+ + H) calcd 1214.6569, found 1214.6587.

To a solution of 6-(benzyloxycarbonylamino)hexyl 2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-pivaloyl-α-D-mannopyranoside (408 mg, 0.34 mmol) in THF (1.5 mL) was added 1.0 M NaOH/MeOH solution (2 mL). The mixture was allowed to stir at room temperature for 20 h and then quenched with sat. NH_4Cl (5 mL). The reaction mixture was extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by flash silica column chromatography (20 → 40% EtOAc/hexanes) afforded **6** (289 mg, 76%) as a clear oil. $[\alpha]^{24}_D$: +25.6° (*c* 1.0, CH_2Cl_2); IR (thin film) 3349, 2924, 1718, 1496, 1454 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.20 (m, 35H), 5.10 (s, 2H), 4.97 (d, J = 11.6 Hz, 1H), 4.92 (d, J = 11.3 Hz, 1H), 4.86 (d, J = 1.2 Hz, 1H), 4.79 (d, J = 11.3 Hz, 1H), 4.74 (d, J = 11.0 Hz, 1H), 4.72 (d, J = 11.9 Hz, 1H), 4.68 (d, J = 11.9 Hz, 1H), 4.63 (d, J = 11.6 Hz, 1H), 4.59−4.54 (m, 2H), 4.45 (d, J = 7.9 Hz, 1H), 4.40 (d, J = 12.2 Hz, 1H), 4.38 (d, J = 11.6 Hz, 1H) 4.27 (d, J = 11.6 Hz, 2H), 4.14−4.10 (m, 1H), 4.00 (s, 1H), 3.91 (d, J = 2.7 Hz, 1H), 3.83−3.66 (m, 8H), 3.61−3.57 (m, 1H), 3.46−3.37 (m, 5H), 3.16 (q, J = 6.7 Hz, 2H), 2.51 (d, J = 1.2 Hz, 1H) 1.59−1.34 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 156.3, 138.9, 138.8, 138.7, 138.5, 138.0, 136.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 127.8, 127.7, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 103.1, 98.9, 82.5, 79.9, 78.1, 77.4, 75.1, 74.6, 74.3, 73.5, 73.4, 73.1, 73.0, 72.7, 72.6, 71.0, 69.4, 68.6, 68.4, 67.5, 66.6, 41.0, 29.9, 29.7, 29.2, 26.5, 25.8, 14.1; FAB MS m/z (M^+ + H) calcd 1130.5993, found 1130.5968.

6-(Benzyloxycarbonylamino)hexyl-(2-*O*-pivaloyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside 8. A mixture of **7** (110 mg, 0.19 mmol) and disaccharide acceptor **6** (143 mg, 0.13 mmol) was azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. Freshly dried 4 Å molecular sieves (110 mg) and CH_2Cl_2 (3 mL) were added. The suspension was cooled to 0 °C, and methyl triflate (60 μL, 0.53 mmol) was added dropwise. After stirring at 0 °C for 10 h, the reaction mixture was warmed to room temperature, and triethylamine (1 mL) was added. The reaction was diluted with EtOAc (20 mL), filtered, and washed with sat. $NaHCO_3$ (2 × 30 mL) and brine (1 × 30 mL). Following drying (Na_2SO_4) and concentration, the crude product was purified by flash silica column chromatography (20 → 30% EtOAc/hexanes) to afford **8** (136 mg, 66%) as a clear oil. $[\alpha]^{24}_D$: +19.7° (*c* 1.0, CH_2Cl_2); IR (thin film) 2926, 1731, 1496, 1453, 1364 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.20 (m, 50H), 5.52−5.51 (m, 1H), 5.01 (s, 2H), 5.04 (d, J = 1.5 Hz, 1H), 4.91 (d, J = 11.9 Hz, 1H), 4.86 (d, J = 11.6 Hz, 1H), 4.82−4.78 (m, 3H), 4.70 (d, J = 11.3 Hz, 1H), 4.66 (d, J = 11.6 Hz, 1H), 4.64−4.59 (m, 3H), 4.53−4.48 (m, 4H), 4.44 (d, J = 11.3 Hz, 1H), 4.39 (d, J = 12.2 Hz, 1H), 4.31 (d, J = 3.7 Hz, 1H), 4.29 (d, J = 2.7 Hz, 1H), 4.20 (d, J = 11.6 Hz, 1H), 4.12−4.08 (m, 1H), 4.00−3.92 (m, 4H), 3.88−3.85 (m, 2H), 3.80−3.69 (m, 8H), 3.64−3.55 (m, 1H), 3.53 (t, J = 8.5 Hz, 1H), 3.46−3.36 (m, 4H), 3.31−3.27 (m, 2H), 3.14−3.11 (m, 2H), 1.52−1.26 (m, 12H), 1.15 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.6, 157.1, 139.9, 139.6, 139.5, 139.4, 139.1, 139.1, 138.7, 137.4, 129.2, 129.0, 128.9, 128.9, 128.9, 128.8, 128.8, 128.7, 128.5, 128.4, 128.4, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 128.0, 128.0, 127.6, 127.5, 104.1, 100.0, 99.3, 83.4, 80.6, 79.2, 78.7, 77.9, 76.1, 75.8, 75.7, 75.6, 75.2, 74.9, 74.0, 73.9, 73.8, 73.7, 73.4, 73.1, 73.0, 72.4, 72.3, 72.0, 69.9, 68.7, 68.5, 68.2, 67.5, 41.7, 39.5, 39.4, 34.7, 30.6, 30.4, 30.0, 29.6, 27.9, 27.7, 26.6, 25.2; FAB MS m/z (M^+ + Na^+) calcd 1668.8325, found 1668.8298.

6-(Benzyloxycarbonylamino) Hexyl-(2-*O*-pivaloyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside 9. To a solution of **8** (130 mg, 0.078 mmol) in THF (500 μL) was added 1.0 M NaOH/MeOH solution (500 μL). The mixture was allowed to stir at room temperature for 17 h and quenched with sat. NH_4Cl (5 mL). Following extraction with EtOAc (3 × 5 mL), the combined extracts were washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by flash silica column chromatography (20 → 40% EtOAc/hexanes) afforded 6-(benzyloxycarbonylamino)hexyl-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (93.3 mg, 76%) as a clear oil. $[\alpha]^{24}_D$: +24.8° (*c* 1.0, CH_2Cl_2); IR (thin film) 2924, 1718, 1496, 1453, 1363 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.18 (m, 50H), 5.14−5.13 (m, 1H), 5.09 (s, 2H), 4.94 (d, J = 11.3 Hz, 1H), 4.88 (d, J = 11.9 Hz, 1 H), 4.85 (d, J = 2.1 Hz, 1H), 4.79 (d, J = 11.0 Hz, 1H), 4.76 (t, J = 11.3 Hz, 2H), 4.69 (d, J = 11.9 Hz, 1H), 4.65 (d, J = 11.9 Hz, 1H), 4.64−4.60 (m, 3H), 4.56 (d, J = 10.1 Hz, 1H), 4.50−4.48 (m, 3H), 4.46−4.38 (m, 4H), 4.33 (d, J = 11.9 Hz, 1H), 4.22 (d, J = 11.9 Hz, 1H), 4.12 (t, J = 9.2 Hz, 1H), 4.05−4.00 (m, 2H), 3.92−3.66 (m, 11H), 3.60−3.58 (m, 1H), 3.54−3.50 (m, 1H), 3.44 (dd, J = 3.1, 9.8 Hz, 1H), 3.41−3.37 (m, 2H), 3.29−3.26 (m, 1H), 3.12 (q, J = 6.7 Hz, 2H), 1.91−1.62 (br s, 1H), 1.51−1.26 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 156.5, 139.4, 139.1, 139.0, 138.9, 138.6, 138.6, 138.5, 138.2, 138.2, 136.9, 128.7, 128.7, 128.6, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.3, 128.1, 128.1, 128.0, 128.0, 128.0, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.3, 103.3, 101.0, 98.9, 82.8, 80.2, 80.1, 78.1, 77.4, 76.8, 75.5, 75.4, 75.2, 75.1, 74.8, 74.6, 73.8, 73.6, 73.6, 73.6, 73.4, 73.2, 72.9, 72.8, 72.1, 71.9, 71.7, 69.4, 69.2, 68.8, 68.5, 67.7, 66.7, 41.2, 30.1, 29.9, 29.5, 26.7, 26.1; FAB MS m/z (M^+ + Na^+) calcd 1570.7224, found 1570.7176. A mixture of **7** (108 mg, 0.19 mmol) and trisaccharide acceptor 6-(benzyloxycarbonylamino)hexyl-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-*β*-D-galactopy-

ranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (193 mg, 0.12 mmol) were azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. Freshly dried 4 Å molecular sieves (100 mg) and CH_2Cl_2 (3 mL) were added. The suspension was cooled to 0 °C, and methyl triflate (60 μL, 0.53 mmol) was added dropwise. After stirring at 0 °C for 10 h, the reaction mixture was warmed to room temperature, and triethylamine (1 mL) was added. The reaction was diluted with EtOAc (20 mL), filtered, and washed with sat. $NaHCO_3$ (2 × 30 mL) and brine (1 × 30 mL). Following drying (Na_2SO_4), filtration and concentration the crude product was purified by silica gel chromatography (15 → 20% EtOAc/hexanes) to afford **9** (180 mg, 70%) as a clear oil. $[\alpha]^{24}_D$: +14.8° (*c* 1.0, CH_2Cl_2); IR (thin film) 2922, 1730, 1454, 1362, 1108 cm^{-1};^{1}H NMR ($CDCl_3$) δ 7.36−7.12 (m, 75H), 5.49−5.48 (m, 1H), 5.09 (s, 2H), 4.93 (d, *J* = 11.3 Hz, 1H), 4.90 (dd, *J* = 1.8, 9.7 Hz, 1H), 4.83−4.76 (m, 4H), 4.73 (s, 1H), 4.72−4.62 (m, 6H), 4.58−4.53 (m, 6H), 4.51 (s, 2H), 4.48−4.40 (m, 6H), 4.36 (d, *J* = 11.0 Hz, 1H), 4.28 (d, *J* = 12.2 Hz, 1H), 4.23 (d, *J* = 11.6 Hz, 1H), 4.16 (d, *J* = 11.6 Hz, 1H), 4.12−4.08 (m, 1H), 4.01 (s, 1H), 3.98−3.95 (m, 3H), 3.90 (d, *J* = 2.7, 1H), 3.88−3.85 (m, 2H), 3.82−3.78 (m, 4H), 3.76−3.61 (m, 6H), 3.58−3.55 (m, 3H), 3.46−3.43 (m, 3H), 3.36 (q, *J* = 4.9 Hz, 2H), 3.26−3.22 (m, 2H), 3.14−3.10 (m, 2H), 1.54−1.26 (m, 12H), 1.18 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.9, 157.0, 140.0, 139.7, 139.5, 139.5, 139.3, 139.2, 139.2, 139.2, 139.1, 139.1, 139.1, 138.6, 137.4, 129.2, 129.0, 129.0, 129.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.6, 128.6, 128.5, 128.4, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.8, 127.7, 103.6, 101.7, 100.0, 99.2, 83.4, 80.8, 80.4, 78.9, 78.4, 75.9, 75.8, 75.7, 75.7, 75.5, 75.5, 75.4, 75.3, 74.8, 74.0, 73.9, 73.7, 73.7, 73.4, 73.2, 73.0, 72.9, 72.7, 72.6, 72.3, 72.1, 70.3, 69.7, 69.6, 69.0, 68.8, 68.2, 67.3, 54.2, 41.7, 39.6, 30.6, 30.4, 30.1, 27.9, 27.7, 27.2, 26.6; FAB MS *m/z* ($M^+ + Na^+$) calcd 2086.9736, found 2087.0706.

6-(amino)hexyl-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→2)-*O*-[(β-D-galactopyranosyl)-(1→4)]-α-D-mannopyranoside 10. Tetrasaccharide **9** (181 mg, 0.087 mmol) was dissolved in THF (500 μL), and a 1.0 M solution of NaOH in MeOH (500 μL) was added. The mixture was allowed to stir at room temperature for 31 h and quenched with sat. NH_4Cl (5 mL). The reaction mixture was extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. Purification by flash silica column chromatography (20 → 40% EtOAc/hexanes) afforded 6-benzyloxycarbonylamino) hexyl-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (85.5 mg, 50%) as a clear oil. $[\alpha]^{24}_D$: +23.6° (*c* 1.0, CH_2Cl_2); IR (thin film) 2923, 2360, 1716, 1454, 1362 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.01 (m, 65H), 5.09 (s, 2H), 5.03 (d, *J* = 1.8 Hz, 1H), 4.91 (d, *J* = 11.0 Hz, 2H), 4.87 (d, *J* = 10.1 Hz, 1H), 4.83 (d, *J* = 9.8 Hz, 1H), 4.81 (d, *J* = 11.3 Hz, 1H), 4.78−4.68 (m, 4H), 4.66−4.58 (m, 4H), 4.56−4.46 (m, 9H), 4.43−4.39 (m, 3H), 4.33 (d, *J* = 11.0 Hz, 1H), 4.29−4.24 (m, 4H), 4.19 (d, *J* = 11.6 Hz, 1H), 3.96−3.90 (m, 4H), 3.89−3.84 (m, 5H), 3.78−3.64 (m, 10H), 3.61−3.55 (m, 4H), 3.41 (dd, *J* = 2.7, 9.8 Hz, 1H), 3.33−3.25 (m, 4H), 3.14−3.11 (m, 4H), 1.50−1.11 (m, 14H); ^{13}C NMR ($CDCl_3$) δ 157.1, 139.9, 139.7, 139.5, 139.5, 139.3, 139.2, 139.2, 139.0, 138.8, 138.7, 137.4, 129.5, 129.3, 129.3, 129.2, 129.2, 129.2, 129.2, 129.2, 129.1, 129.1, 129.0, 129.0, 129.0, 129.0, 128.9, 128.9, 128.8, 128.7, 128.6, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.8, 103.6, 101.7, 99.3, 83.4, 80.8, 80.6, 80.3, 78.5, 78.3, 78.0, 78.0, 77.8, 77.5, 76.0, 75.9, 75.7, 75.7, 75.6, 75.5, 75.3, 75.0, 74.2, 74.0, 73.9, 73.9, 73.7, 73.5, 73.4, 73.2, 73.0, 72.8, 72.8, 72.2, 72.2, 70.3, 69.7, 69.6, 69.2, 69.0, 68.2, 68.0, 67.3, 41.7, 30.6, 30.1, 27.2, 26.6; FAB MS *m/z* ($M^+ + H$) 1994.9897, found 1994.9816. 10% Pd/C (50 mg) was suspended in ethanol (10 mL). The flask was purged with H_2, and a hydrogen balloon was attached. After 20 min, tetrasaccharide (benzyloxycarbonylamino) hexyl-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-[(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (40 mg, 0.02 mmol) in EtOAc (1 mL) was added dropwise. After 48 h, the solution was filtered and concentrated. Lyophilization (H_2O) afforded the leishmania cap tetrasaccharide **10** (9 mg, 53%) as a white powder. $[\alpha]^{24}_D$: +38.7° (*c* 0.71, MeOH); IR (thin film) 3383, 2928, 1653, 1127, 1063 cm^{-1}; ^{1}H NMR (D_2O) δ 7.41 (s, 1H), 5.29 (d, *J* = 1.2 Hz, 1H), 5.09 (d, *J* = 1.2 Hz, 1H), 5.01 (d, *J* = 1.5 Hz, 1H), 4.41 (d, *J* = 7.9 Hz, 1H), 4.11 (dd, *J* = 1.8, 3.1 Hz, 1H), 4.05−4.04 (m, 1H), 4.00−3.50 (m, 24H), 2.99−2.95 (m, 2H), 1.65−1.60 (m, 5H), 1.38 (br s, 4H); ^{13}C NMR (D_2O) δ 103.7, 102.8, 101.2, 98.4, 79.3, 79.1, 77.3, 75.9, 73.8, 73.1, 72.0, 71.6, 70.9, 70.5, 70.5, 69.6, 69.2, 68.6, 67.7, 67.5, 61.8, 61.8, 61.7, 60.9, 40.0, 28.8, 27.2, 26.0, 25.5; FAB MS *m/z* ($M^+ + H$) calcd 766.3345, found 766.3327.

6-(*N*-Palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*R*,2*S*)-propyl]-L-cystenyl)hexyl-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→2)-*O*-[(β-D-galactopyranosyl)-(1→4)]-α-D-mannopyranoside 1. Amino-tetrasaccharide **10** (8.6 mg, 11.2 μmol), palmitoyl-cysteinyl ((*RS*)-2,3-di(palmitoyloxy)-propyl)-OH **11** (26 mg, 28.5 μmol), and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (8.5 mg, 22.4 μmol) were dissolved in 2.25 mL of CH_2Cl_2:DMF (2:1). Diisopropylethylamine (5 μL, 28.5 μmol) was added, and the resulting yellow solution was allowed to stir at room temperature for 20 h. The mixture was concentrated in vacuo, and purified by silica gel column chromatography (2→50% MeOH/CH_2Cl_2) to afford **1** (11.2 mg, 60%) as a white powder. ^{1}H NMR ($CDCl_3/D_2O$) δ 8.61 (s, 1H), 8.31 (d, *J* = 7.9 Hz, 1H), 7.39−7.19 (m, 2H), 5.23−4.73 (m, 4H), 4.18−2.74 (m, 43H), 2.38−2.12 (m, 12H), 1.58−1.42 (m, 13H), 1.26−1.00 (s, 98H), 0.89−0.86 (m, 14H); FAB MS *m/z* ($M^+ + Na^+$) calcd 1680.0513, found 1680.0468; MALDI-TOF [M + Na]$^+$ 1681.

6-(Amino)hexyl-(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→2)-*O*-[(β-D-galactopyranosyl)-(1→4)]-α-D-mannopyranoside-KLH 2. A solution of *N*-succidimidyl bromoacetate (3.5 mg, 15 μmol) in anhydrous DMF (250 μL) was mixed with a solution of KLH (11.8 mg) in 2 mL of 1 x PBS, pH 7.4. After 2 h, the reaction mixture was subjected to gel filtration using a Pd-10 column that was equilibrated with 1 x PBS, pH 7.4. The protein was collected in 3.5 mL eluent. The solution of bromoacetylated KLH (3.5 mL) thus obtained was added to the *S*-acetylmercaptoacetyl-modified carbohydrate **12** (1.7 mg, 1.92 μmol) and incubated with 100 mL of 0.2 M hydroxylamine. After 24 h, the remaining bromoacetyl groups were blocked by the addition of 2-aminoethanethiol (3 mg, 20 μmol). After an additional 24 h, the mixture was diluted up to 5 mL with 1 x PBS and run on two Pd-10 columns. The protein was collected in 3.5 mL of eluent (for each column). Any nonconjugated carbohydrate was removed on a Centriprep YM-30 filter (Millipore, MWCO 30,000). The concentrated sugar−KLH conjugate **2** was stored at 4 °C.

***p*-Methoxyphenyl 2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranoside 15.** Dihydroxy mannoside **14**[33] (1.15 g, 2.47 mmol) was dissolved in pyridine (20 mL) and cooled to −20 °C. Benzoyl chloride (745 μL, 6.41 mmol) was added, and the mixture was stirred at −20 °C. After 15 min TLC analysis showed complete consumption of the starting material. Methanol (3 mL) was added, and the solution was allowed to warm to room temperature and then diluted with EtOAc. The organic layer was separated and washed with H_2O (2 × 50 mL), sat. $NaHCO_3$ (2 × 50 mL), and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (20 → 30% EtOAc/hexanes) gave *p*-methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside as a clear oil (1.13 g, 80%). $[\alpha]^{24}_D$: +11.7° (*c* 0.29, CH_2Cl_2); IR (thin film) 1721, 1507, 1452, 1362, 1270 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.08 (dd, *J* = 1.2, 8.2 Hz, 2H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.6 Hz, 2H), 7.42−7.26 (m, 9H), 7.06 (d, *J* = 9.2 Hz, 2H), 6.81 (d, *J* = 9.2 Hz, 2H), 5.78 (dd, *J* = 2.1, 3.1 Hz, 1H), 5.59 (d, *J* = 1.8 Hz, 1H), 4.87 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 11.9 Hz, 1H), 4.59 (d, *J* = 11.0 Hz, 1H), 4.56 (d, *J* = 11.9 Hz, 1H), 4.26 (td, *J* = 1.5, 9.5 Hz, 1H), 4.12 (dd, *J* = 3.4, 9.5 Hz, 1H), 4.06−4.02 (m, 1H), 3.87 (dd, *J* = 4.6, 10.7 Hz, 1H), 3.81 (dd, *J* = 2.7, 10.7 Hz, 1H), 3.78 (s, 3H), 2.60 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 165.5, 155.0, 149.8, 138.1, 137.5, 133.2, 129.8, 129.4, 128.4,

128.3, 128.2, 128.0, 127.8, 127.4, 127.3, 117.9, 114.5, 97.2, 77.5, 73.5, 71.9, 69.6, 68.2, 67.1, 55.6; FAB MS *m/z* ($M^+ + Na^+$) calcd 593.2151, found 593.2145.To a solution of *p*-methoxyphenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (1.56 g, 2.73 mmol) in CH_2Cl_2 (27 mL) was added 2,6-lutidine (794 μL, 6.82 mmol), followed by the addition of *tert*-butyldimethylsilyl trifluoromethanesulfonate (941 μL, 4.10 mmol). After 20 min, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (20 → 30% EtOAc/hexanes) yielded **15** as an oil (1.79 g, 96%). $[\alpha]^{24}_D$: +30.0° (*c* 4.08, CH_2Cl_2); IR (thin film) 3063, 2855, 1724, 1507, 1360 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.06−8.05 (m, 2H), 7.61−7.56 (m, 2H), 7.42−7.21 (m, 14H), 7.10 (dd, *J* = 2.5, 9.1 Hz, 2H), 6.83 (dd, *J* = 2.2, 9.1 Hz, 2H), 5.78 (dd, *J* = 2.2, 3.0 Hz, 1H), 5.58 (d, *J* = 1.9 Hz, 1H), 4.86 (d, *J* = 11.0 Hz, 1H), 4.65 (d, *J* = 11.8 Hz, 1H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.54 (d, *J* = 10.3 Hz, 1H), 4.32 (t, *J* = 9.3 Hz, 1H), 4.10−4.02 (m, 2H), 3.91 (dd, *J* = 4.7, 10.7 Hz, 1H), 3.85−3.78 (m, 4H), 0.86 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.6, 155.1, 150.2, 138.6, 137.7, 133.1, 129.8, 129.8, 128.4, 128.1, 128.0, 127.7, 127.3, 127.2, 127.1, 118.1, 114.5, 97.1, 78.0, 73.4, 73.0, 71.0, 69.3, 68.3, 67.6, 55.5, 25.9, 18.2, −3.9, −5.2; FAB MS *m/z* ($M^+ + Na^+$) calcd 707.3016, found 707.3029.

2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-*tert*-butyldimethylsilyl-α-D-mannopyranose Trichloroacetimidate 16. To a solution of mannoside **15** (22.1 mg, 0.032 mmol) in CH_3CN/H_2O (1 mL, 4/1) was added cerium ammonium nitrate (53 mg, 0.096 mmol). The resulting clear orange solution was stirred at room temperature for 10 min and then loaded onto a silica column. Elution with 30% EtOAc/hexanes gave 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-*tert*-butydimethylsilyl-α-D-mannopyranose as an orange oil (18.4 mg, 99%). $[\alpha]^{24}_D$: −28.6° (*c* 1.51, CH_2Cl_2); IR (thin film) 3421, 3927, 1723, 1452, 1271 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.03 (dd, *J* = 1.2, 8.5 Hz, 1H), 7.59−7.56 (m, 1H), 7.42−7.19 (m, 12H), 5.58 (dd, *J* = 2.1, 3.1 Hz, 1H), 5.37 (s, 1H), 4.75 (d, *J* = 11.3 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.60 (d, *J* = 12.2 Hz, 1H), 4.44 (d, *J* = 11.0 Hz, 1H), 4.12−4.08 (m, 1H), 4.05−4.02 (m, 1H), 3.91 (dd, *J* = 3.1, 8.9 Hz, 1H), 3.80 (dd, *J* = 1.83, 10.1 Hz, 1H), 3.75−3.71 (m, 1H), 3.16 (s, 1H), 0.79 (s, 9H), −0.04 (s, 3H), −0.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.9, 138.3, 133.0, 130.1, 130.0, 128.6, 128.6, 128.2, 128.0, 127.9, 127.8, 127.5, 92.7, 77.9, 77.5, 73.6, 73.0, 71.2, 69.1, 68.5, 26.3, 18.5, −3.5, −4.8; FAB MS *m/z* ($M^+ + Na^+$) calcd 601.2597, found 601.2608.To a solution of 2-*O*-benzoyl-3,6-di-*O*-benzyl-4-*O*-*tert*-butydimethylsilyl-α-D-mannopyranose (100 mg, 0.173 mmol) in CH_2Cl_2 (1 mL) at −20 °C was added Cl_3CCN (173 μL, 1.73 mmol), followed by DBU (3 μL, 0.017 mmol). The resulting dark green solution was stirred for 5 min and then filtered through a pad of silica. The silica was washed with 40% EtOAc/hexanes (100 mL), and the combined washes were concentrated to yield **16** as an orange oil (87 mg, 70%). $[\alpha]^{24}_D$: +7.1° (*c* 2.51, CH_2Cl_2); IR (thin film) 2928, 1728, 1674, 1263, 1105 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.71 (s, 1H), 8.05 (dd, *J* = 1.2, 8.2 Hz, 2H), 7.59−7.56 (m, 1H), 7.39−7.21 (m, 14H), 6.40 (d, *J* = 2.1 Hz, 1H), 5.71 (dd, *J* = 2.1, 3.1 Hz, 1H), 4.77 (d, *J* = 11.3 Hz, 1H), 4.67 (d, *J* = 11.9 Hz, 1H), 4.61 (d, *J* = 11.9 Hz, 1H), 4.51 (d, *J* = 11.3 Hz, 1H), 4.37−4.33 (m, 1H), 4.00−3.97 (m, 1H), 3.93−3.90 (m, 1H), 3.79−3.77 (m, 1H), 0.84 (s, 9H), 0.04 (s, 3H), −0.01 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.3, 159.9, 138.6, 137.3, 133.2, 129.9, 129.5, 128.4, 128.2, 128.1, 127.5, 127.2, 127.1, 95.3, 90.9, 77.5, 76.1, 73.2, 71.2, 68.7, 67.1, 67.0, 26.0, 25.9, 18.2, −3.9, −5.2; FAB MS *m/z* ($M^+ + Na^+$) calcd 601.2597, found 601.2608.

***n*-Pentenyl 2-*O*-Benzoyl-3,6-di-*O*-benzyl-4-*O*-*tert*-butydimethylsilyl-α-D-mannopyranoside 19.** To a solution of **16** (77 mg, 0.11 mmol) and 4-penten-1-ol (12 μL, 0.12 mmol) in CH_2Cl_2(1 mL) was added TBSOTf (1.3 μL, 0.01 mmol). The mixture was stirred at room temperature for 1 h, diluted with CH_2Cl_2 (50 mL), washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (20 → 30% EtOAc/hexanes) yielded **19** (18 mg, 25%; 76% based on recovered desilylated product) as an oil. $[\alpha]^{24}_D$: −38.5° (*c* 0.13, CH_2Cl_2); IR (thin film) 2926, 2360, 1724, 1267, 1109 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.05−8.03 (m, 2H), 7.58−7.55 (m, 2H), 7.40−7.19 (m, 14H), 5.89−5.81 (m, 1H), 5.58 (t, *J* = 2.1 Hz, 1H), 5.09−5.01 (m, 2H), 4.96 (s, 1H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.68−4.63 (m, 2H), 4.45 (d, *J* = 11.0 Hz, 1H), 4.19−4.15 (m, 1H), 3.87−3.76 (m, 5H), 3.48 (dt, *J* = 6.1, 9.5 Hz, 1H), 2.19−2.15 (m, 2H), 1.78−1.72 (m, 2H), 0.82 (s, 9H), 0.0 (s, 3H), −0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.5, 139.5, 138.7, 138.6, 133.8, 130.7, 130.5, 129.1, 129.0, 128.7, 128.5, 128.0, 127.9, 115.7, 98.4, 78.9, 77.9, 73.9, 73.7, 71.6, 70.2, 69.3, 68.6, 68.0, 31.0, 29.3, 26.7, 18.9, −3.2, −4.5; FAB MS *m/z* ($M^+ + Na^+$) calcd 669.3218, found 669.3226.

***n*-Pentenyl 3,4,6-Tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside 22.** To a solution of **19** (18 mg, 0.028 mmol) in THF (1 mL) was added a 1.0 M solution of TBAF in THF (56 μL, 0.056 mmol). The resulting orange solution was stirred at room temperature for 1.5 h and then purified by silica gel column chromatography (30% EtOAc/hexanes) to afford *n*-pentenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (14.4 mg, 97%) as a yellow oil. $[\alpha]^{24}_D$: −21.8° (*c* 0.19, CH_2Cl_2); IR (thin film) 3488, 2920, 1721, 1452, 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.07−8.05 (m, 2H), 7.58−7.56 (m, 1H), 7.42−7.25 (m, 1H), 5.87−5.79 (m, 1H), 5.60−5.59 (m, 1H), 5.08−4.98 (m, 2H), 4.81 (d, *J* = 11.3 Hz, 1H), 4.70 (d, *J* = 11.9 Hz, 1H), 4.61 (d, *J* = 12.2 Hz, 1H), 4.51 (d, *J* = 11.0 Hz, 1H), 4.15 (t, *J* = 9.2 Hz, 1H), 3.91 (dd, *J* = 3.4, 9.5 Hz, 1H), 3.89−3.81 (m, 3H), 3.79−3.74 (m, 1H), 3.48 (dt, *J* = 6.4, 9.8 Hz, 1H), 2.52 (s, 1H), 2.18−2.13 (m, 2H), 1.76−1.70 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.5, 139.0, 138.6, 138.4, 133.9, 130.6, 130.4, 129.2, 129.1, 129.0, 128.8, 128.6, 128.1, 115.8, 98.7, 78.4, 74.3, 72.1, 70.6, 69.1, 68.0, 68.0, 31.0, 29.3; FAB MS *m/z* ($M^+ + Na^+$) calcd 555.2353, found 555.2356. A mixture of galactosyl phosphate **20** (25 mg, 0.034 mmol) and acceptor *n*-pentenyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-mannopyranoside (15 mg, 0.029 mmol) were azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. The mixture was dissolved in CH_2Cl_2 (1 mL) and cooled to −78 °C. Following the addition of TMSOTf (6.3 μL, 0.034 mmol), the solution was stirred at −78 °C for 40 min and then triethylamine (3 mL) was added. After warming to room temperature, the reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (20 → 30% EtOAc/hexanes) yielded **22** (20 mg, 66%) as an oil. $[\alpha]^{24}_D$: −4.9° (*c* 0.34, CH_2Cl_2); IR (thin film) 2924, 2359, 1723, 1454, 1268 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98 (d, *J* = 8.2 Hz, 2H), 7.48−7.47 (m, 2H), 7.34−7.22 (m, 37H), 7.15−7.11 (m, 5H), 5.84−5.76 (m, 1H), 5.54 (d, *J* = 1.8 Hz, 1H), 5.43−5.39 (m, 1H), 5.04−4.96 (m, 2H), 4.93−4.85 (m, 2H), 4.81 (d, *J* = 12.2 Hz, 1H), 4.72 (s, 2H), 4.68−4.62 (m, 2H), 4.51 (d, *J* = 11.9 Hz, 1H), 4.47 (t, *J* = 11.9 Hz, 2H), 4.31−4.24 (m, 3H), 4.05 (dd, *J* = 2.7, 9.2 Hz, 1H), 3.91−3.82 (m, 4H), 3.76 (d, *J* = 10.4 Hz, 1H), 3.72−3.68 (m, 1H), 3.57−3.54 (m, 1H), 3.47−3.37 (m, 4H), 2.13−2.08 (m, 2H), 1.71−1.65 (m, 2H), 1.15 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.5, 166.5, 139.4, 139.3, 139.2, 138.6, 138.5, 133.6, 130.7, 130.5, 129.0, 128.9, 128.8, 128.7, 128.7, 128.5, 128.3, 128.3, 128.2, 128.1, 127.7, 115.7, 100.9, 98.3, 81.9, 77.9, 76.9, 75.1, 74.2, 74.1, 73.0, 72.8, 72.6, 71.9, 70.6, 69.8, 69.1, 68.0, 39.5, 30.9, 29.2, 28.0; FAB MS *m/z* ($M^+ + Na^+$) calcd 1071.4871, found 1071.4854.

***n*-Pentenyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-[(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside 25.** To a solution of **22** (23 mg, 0.022 mmol) in 4:1 CH_2Cl_2:MeOH (1 mL) was added a 0.5 M solution of NaOMe in MeOH (260 μL, 0.13 mmol). After being stirred at room temperature for 1 h, the crude mixture was purified by flash silica column chromatography (20 → 40% EtOAc/hexanes) to afford *n*-pentenyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-benzyl-α-D-mannopyranoside (18.8 mg, 90%) as an oil. $[\alpha]^{24}_D$: +35.0° (*c* 0.22, CH_2Cl_2); IR (thin film) 2871, 1740, 1454, 1366, 1067 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40−7.18 (m, 30H), 5.83−5.75 (m, 1H), 5.40−5.36 (m, 1H), 5.03−4.96 (m, 2H), 4.95−4.91 (m, 3H), 4.84 (d, *J* = 1.2 Hz, 1H), 4.78 (d, *J* = 12.2 Hz, 1H), 4.66 (d, *J* = 11.9 Hz, 1H), 4.58 (d, *J* = 11.0 Hz, 1H), 4.50−4.47 (m, 2H), 4.46−4.42 (m, 2H),

4.42 (d, J = 11.9 Hz, 1H), 4.28 (d, J = 11.9 Hz, 1H), 4.14 (t, J = 9.5 Hz, 1H), 3.98−3.93 (m, 2H), 3.80−3.72 (m, 2H), 3.69−3.64 (m, 3H), 3.61−3.57 (m, 1H), 3.48 (dd, J = 5.5, 9.2 Hz, 1H), 3.44−3.41 (m, 2H), 3.33 (dd, J = 2.8, 10.1 Hz, 1H), 2.18−2.02 (m, 2H), 1.66−1.58 (m, 2H), 1.15 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.1, 138.8, 138.8, 138.2, 138.0, 128.6, 128.6, 128.4, 128.3, 128.3, 128.2, 128.0, 128.0, 128.0, 127.8, 127.8, 127.6, 127.5, 127.3, 100.4, 99.2, 81.2, 78.0, 77.6, 74.7, 73.7, 73.6, 73.3, 72.1, 71.0, 69.7, 67.2, 39.0, 30.5, 28.8, 27.5; FAB MS m/z ($M^+ + Na^+$) calcd 967.4608, found 967.4594. A mixture of mannosyl trichloroacetimidate **23** (50 mg, 0.078 mmol) and *n*-pentenyl 3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-*β*-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-α-D-mannopyranoside acceptor (37 mg, 0.039 mmol) was azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. The mixture was dissolved in CH_2Cl_2 (1 mL), and a 0.5 M solution of TMSOTf in CH_2Cl_2 (4 μL, 0.002 mmol) was added. The solution was stirred at room temperature for 5 min and then diluted with CH_2Cl_2 (50 mL), washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (10 → 30% EtOAc/hexanes) yielded **25** (37 mg, 67%) as an oil. $[\alpha]^{24}_D$: +17.7° (*c* 1.0, CH_2Cl_2); IR (thin film) 2920, 1741, 1453, 1368, 1235 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.37−7.12 (m, 45H), 5.81−5.74 (m, 1H), 5.56 (dd, J = 1.8, 3.1 Hz, 1H), 5.39 (dd, J = 8.2, 10.1 Hz, 1H), 5.14 (d, J = 1.5 Hz, 1H), 5.03−4.99 (m, 1H), 4.96−4.94 (m, 2H), 4.85 (d, J = 11.6 Hz, 1H), 4.82−4.78 (m, 3H), 4.69−4.56 (m, 5H), 4.50−4.42 (m, 6H), 4.37 (d, J = 11.9 Hz, 1H), 4.28 (d, J = 11.6 Hz, 1H), 4.24 (d, J = 10.7 Hz, 1H), 4.18−4.14 (m, 1H), 4.04−4.03 (m, 1H), 3.95−3.91 (m, 3H), 3.87 (dd, J = 3.1, 9.2 Hz, 1H), 3.83−3.76 (m, 3H), 3.72−3.69 (m, 3H), 3.64−3.58 (m, 3H), 3.53 (dd, J = 4.9, 8.9 Hz, 1H), 3.45−3.41 (m, 2H), 3.32 (dt, J = 6.4, 9.8 Hz, 1H), 2.09−2.03 (m, 5H), 1.65−1.61 (m, 3H), 1.14 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.6, 170.5, 139.5, 139.4, 139.2, 138.9, 138.8, 138.7, 138.7, 138.6, 129.1, 129.0, 129.0, 129.0, 129.0, 128.9, 128.8, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.0, 127.7, 127.7, 127.6, 115.6, 101.2, 100.1, 99.4, 81.8, 79.3, 78.6, 78.0, 75.7, 75.6, 75.0, 74.9, 74.5, 74.1, 74.0, 74.0, 73.7, 73.3, 72.9, 72.6, 72.5, 72.1, 69.8, 69.6, 69.0, 68.8, 67.7, 39.5, 31.0, 29.3, 28.0, 21.8; FAB MS m/z ($M^+ + Na^+$) calcd 1441.6645, found 1441.6684.

***n*-Pentenyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-[(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside 27.** To a solution of **25** (37 mg, 0.026 mmol) in CH_2Cl_2 (1 mL) was added a 0.5 M solution of NaOMe in MeOH (78 μL, 0.039 mmol). After stirring at room temperature for 30 min, the solution was diluted with CH_2Cl_2 (50 mL), washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (10 → 30% EtOAc/hexanes) yielded *n*-pentenyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-[(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside (27 mg, 76%) as an oil. $[\alpha]^{24}_D$: +28.2° (*c* 0.29, CH_2Cl_2); IR (thin film) 2918, 2362, 1740, 1454, 1364 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.13 (m, 40H), 5.80−5.75 (m, 1H), 5.46−5.41 (m, 1H), 5.19 (d, J = 1.8 Hz, 1H), 5.03−4.98 (m, 1H), 4.97−4.92 (m, 3H), 4.85−4.82 (m, 2H), 4.75 (dd, J = 3.1, 11.0 Hz, 1H), 4.67−4.62 (m, 5H), 4.57−4.43 (m, 6H), 4.40−4.38 (m, 2H), 4.35−4.30 (m, 2H), 4.26 (dd, J = 3.1, 11.9 Hz, 1H), 4.23−4.19 (m, 1H), 4.06−4.00 (m, 2H), 3.92−3.79 (m, 5H), 3.77−3.66 (m, 7H), 3.61−3.50 (m, 2H), 3.48−3.43 (m, 3H), 3.32−3.27 (m, 1H), 2.20 (s, 1H), 2.07−2.02 (m, 2H), 1.64−1.57 (m, 2H), 1.16 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.6, 139.8, 139.4, 139.3, 139.1, 139.0, 138.7, 138.7, 138.5, 129.1, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.8, 115.6, 101.6, 100.9, 99.5, 81.8, 80.8, 78.5, 78.0, 76.1, 75.6, 75.1, 75.1, 74.3, 74.2, 74.2, 74.1, 74.1, 74.0, 73.4, 72.7, 72.5, 72.5, 72.4, 72.3, 69.8, 69.6, 69.4, 68.9, 67.7, 39.5, 31.0, 29.3, 28.0; FAB MS m/z ($M^+ + Na^+$) calcd 1399.6540, found 1399.6492. A mixture of **23** (45 mg, 0.072 mmol) and *n*-pentenyl (3,4,6-tri-*O*-benzyl-α-D-mannopyranosly)-(1→2)-[(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside trisaccharide acceptor (50 mg, 0.036 mmol) was azeotroped with toluene (3 × 5 mL) and dried under vacuum for 1 h. The mixture was dissolved in CH_2Cl_2 (1 mL), and a 0.5 M solution of TMSOTf in CH_2Cl_2 (4 μL, 0.002 mmol) was added. The solution was stirred at room temperature for 5 min and then diluted with CH_2Cl_2 (50 mL), washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 50 mL), dried (Na_2SO_4), filtered, and concentrated. Flash silica column chromatography (10 → 30% EtOAc/hexanes) yielded **27** (50 mg, 75%) as an oil. $[\alpha]^{24}_D$: +10.8° (*c* 0.48, CH_2Cl_2); IR (thin film) 2924, 2361, 1741, 1454, 1365 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.36−7.08 (m, 64H), 5.78−5.70 (m, 1H), 5.47−5.46 (m, 1H), 5.40 (dd, J = 7.9, 10.1 Hz, 1H), 5.12 (d, J = 1.5 Hz, 1H), 4.99−4.84 (m, 5H), 4.81−4.74 (m, 5H), 4.71−4.59 (m, 6H), 4.57−4.32 (m, 14H), 4.30−4.17 (m, 4H), 4.13−4.09 (m, 2H), 4.01 (t, J = 2.1 Hz, 1H), 3.99−3.91 (m, 4H), 3.90−3.52 (m, 18H), 3.48−3.35 (m, 5H), 3.26−3.21 (m, 1H), 2.09 (s, 3H), 2.01−1.99 (m, 3H), 1.57−1.53 (m, 8H), 1.12 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 177.6, 170.8, 139.9, 139.4, 139.3, 139.2, 139.1, 139.0, 138.7, 138.6, 138.5, 129.0, 129.0, 128.9, 128.9, 128.8, 128.8, 128.7, 128.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.7, 115.5, 101.6. 100.9, 100.1, 99.3, 81.8, 80.4, 78.7, 78.2, 77.9, 75.8, 75.7, 75.3, 75.1, 74.9, 74.2, 74.0, 73.9, 73.9, 73.7, 73.3, 73.0, 72.8, 72.5, 72.1, 70.2, 69.7, 69.4, 68.9, 67.7, 39.5, 31.0, 30.4, 29.3, 28.0, 21.9; FAB MS m/z ($M^+ + Na^+$) calcd 1873.8588, found 1873.8537.

Solid-Phase Synthesis of *n*-Pentenyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-[(3,4,6-tri-*O*-benzyl-2-*O*-pivaloyl-*β*-D-galactopyranosyl)-(1→4)]-3,6-di-*O*-benzyl-α-D-mannopyranoside 27. *Note: Intermediates were analyzed by removing ca. 2 mg of resin and cleaving from resin using Grubbs catalyst in an atmosphere of ethylene, followed by TLC. Compounds made in solution were used as a reference.* Our functionalized resin (**17**) was prepared in the following manner: Mono-DMT protected octenediol was reacted with Merrifield's resin (chloromethylated polystyrene cross-linked with 1% divinylbenzene), followed by removal of the DMT group by dichloroacetic acid. The resin loading could be determined upon cleavage of the DMT group by a colorimetric assay.[38] Octenediol functionalized Merrifield resin[32] **17** (417 mg, 0.6 mmol/g, 0.25 mmol) was swollen in CH_2Cl_2 (4 mL) and shaken for 15 min. Following addition of donor **16** (542 mg, 0.75 mmol, 3 equiv) in CH_2Cl_2 (4 mL), the flask was shaken for 15 min. A 0.5 M solution of TMSOTf in CH_2Cl_2 (150 μL, 0.075 mmol) was added and shaking continued. After 1 h at room temperature, 10 mL of methanol was added, and the resin was washed with 3 × 5 mL of MeOH, 5 mL of 1:1 MeOH:THF, 3 × 5 mL each; THF and CH_2Cl_2. The glycosylation procedure was repeated, followed by identical washing (double glycosylation). The resin was then dried under vacuum for 12 h. Resin-bound **18** (514 mg, 0.25 mmol) was swollen in THF (8 mL) and shaken for 15 min. Following addition of a 1.0 M solution of TBAF in THF (2.52 mL, 2.52 mmol), the reaction mixture was shaken for 12 h at room temperature. The resin was then washed with 3 × 5 mL of MeOH, 5 mL of MeOH/THF (1:1), 3 × 5 mL of THF, 3 × 5 mL of CH_2Cl_2, and dried under vacuum over P_2O_5 for 12 h prior to glycosylation. Acceptor functionalized resin (233 mg, 0.11 mmol) was swollen in CH_2Cl_2 (3 mL) and shaken for 15 min. Following addition of donor **20** (257 mg, 0.34 mmol, 3 equiv) in CH_2Cl_2 (2 mL), the flask was shaken for 15 min and cooled to −78 °C. A 0.5 M solution of TMSOTf in CH_2Cl_2 (686 μL, 0.34 mmol) was added and shaking continued for 1 h. Upon warming to room temperature, 10 mL of methanol were added, and the resin was washed with 3 × 5 mL of MeOH, 5 mL of MeOH/THF (1:1), 3 × 5 mL each; THF and CH_2Cl_2. The glycosylation procedure was repeated, followed by identical washing (double glycosylation). Benzoyl ester protected carbohydrate bound resin **21** (282 mg, 0.11 mmol) was swollen in CH_2Cl_2 (10 mL) and shaken for 15 min. A 0.5 M solution of NaOMe in MeOH (1.35 mL, 0.68 mmol) was added and

(38) Pon, R. T. In: *Methods in Molecular Biology 20: Protocols for Oligonucleotides and Analogues*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; p 467.

shaking continued for 2 h. The resin was then washed with 3 × 5 mL of 20% MeOH/CH_2Cl_2, 1 × 5 mL of MeOH, 3 × 5 mL each: 20% MeOH/THF, THF and CH_2Cl_2 and dried under vacuum for 12 h. Acceptor-bound resin (239 mg, 0.095 mmol) was swollen in a solution of donor **23** (182 mg, 0.29 mmol, 3 equiv) in CH_2Cl_2 (6 mL) and shaken for 15 min. A solution of 0.5 M TMSOTf in CH_2Cl_2 (58 μL, 0.029 mmol) was added and the reaction mixture was shaken for 1 h at room temperature. The resin was washed with 3 × 5 mL of 20% MeOH/CH_2Cl_2, 1 × 5 mL of MeOH, 3 × 5 mL each: 20% MeOH/THF, THF and CH_2Cl_2. The glycosylation and washing were repeated, and the resin was dried under vacuum for 12 h. Acetate-protected carbohydrate-bound resin **24** (262 mg, 0.094 mmol) was swollen in CH_2Cl_2 (10 mL) and shaken for 15 min. A 0.5 M solution of NaOMe in MeOH (1.13 mL, 0.57 mmol) was added and shaking continued for 2 h. The resin was washed with 3 × 5 mL of 20% MeOH/CH_2Cl_2, 1 × 5 mL of MeOH, 3 × 5 mL each: 20% MeOH/THF, THF and CH_2Cl_2 and dried under vacuum for 12 h. Acceptor bound resin (250 mg, 0.09 mmol) was swollen in a solution of donor **23** (172 mg, 0.27 mmol, 3 equiv) in CH_2Cl_2 (6 mL) and shaken for 15 min. A solution of 0.5 M TMSOTf in CH_2Cl_2 (54 μL, 0.027 mmol) was added, and the reaction mixture was shaken for 1 h at room temperature. The resin was washed with 3 × 5 mL of 20% MeOH/CH_2Cl_2, 1 × 5 mL of MeOH, 3 × 5 mL each: 20% MeOH/THF, THF and CH_2Cl_2. The glycosylation and washing was repeated, and the resin was dried under vacuum for 12 h. Resin-bound tetrasaccharide **26** (281 mg, 0.09 mmol) was swollen in CH_2Cl_2 (6 mL). Grubbs catalyst (15 mg, 0.018 mmol) was added, and the reaction mixture was purged with ethylene and stirred at room temperature for 18 h. Another 10 mol % of the catalyst (15 mg, 0.018 mmol) was added, and the solution was stirred for an additional 18 h. The mixture was diluted with CH_2Cl_2 (100 mL) and passed through a plug of Celite. Following concentration, the crude material was purified by flash silica column chromatography (30 → 40% EtOAc/hexanes) to afford *n*-pentenyl glycoside **27** (29 mg, 18% overall yield).

Acknowledgment. Financial support from: the donors of the Petroleum Research Fund, administered by the ACS (ACS-PRF 34649-G1) for partial support of this research; the NSF (CHE-9808061 and DBI-9729592) for providing NMR facilities; the Mizutani Foundation for Glycoscience and the NIH (Biotechnology Training Grant for M.C.H.) is gratefully acknowledged.

Supporting Information Available: Spectral data for all new compounds is included. This material is available free of charge via the Internet at http://pubs.acs.org.

JO015521Z