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# Synthesis of Galactofuranose-Containing Acceptor Substrates for Mycobacterial Galactofuranosyltransferases

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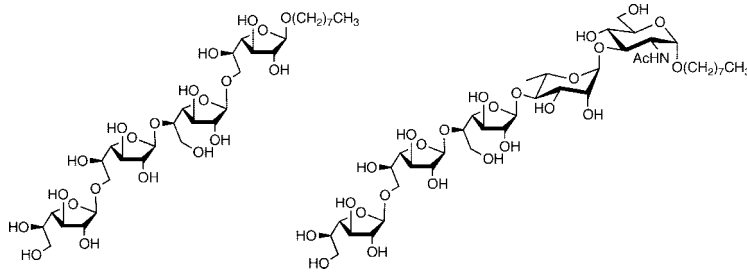
*Received February 26, 2008*



The major structural component of the cell wall in *Mycobacterium tuberculosis*, infection by which causes tuberculosis, is the mycolyl-arabinogalactan (mAG) complex. This large glycoconjugates has at its core a backbone of ~30 D-galactofuranose (Gal*f*) residues that are linked to peptidoglycan by way of a linker disaccharide containing L-rhamnose and 2-acetamido-2-deoxy-D-glucose. Recent studies have supported a model of galactan biosynthesis in which the entire structure is assembled by the action of two bifunctional galactofuranosyltransferases. These biochemical investigations were made possible, in part, by access to a panel of oligosaccharide fragments of the mAG complex (**1**−**12**), the synthesis of which we describe here. An early key finding in this study was that the iodine-promoted cyclization of galactose diethyl dithioacetal (**19**) in the presence of an alcohol solvent led to the formation Gal*f* glycosides contaminated with no pyranoside isomer, thus allowing the efficient preparation of furanoside derivatives of this monosaccharide. The synthesis of disaccharide targets **1**, **2**, **11** and **12** proceeded without difficulty through the use of thioglycoside donors and octyl glycoside acceptors, both carrying benzoyl protection. In the synthesis of the tri- and tetrasaccharides **3**−**6**, we explored routes in which the molecule was assembled from the reducing to nonreducing end, and the reverse. The latter approach was found to be preferable for the preparation of **6**, and in the case of **3** and **4**, this strategy allowed the development of efficient one-pot methods for their synthesis. We have also carried out the first synthesis of three mAG fragments (**8**−**10**) consisting of the linker disaccharide further elaborated with one, two or three Gal*f* residues. A key step in the synthesis of these target compounds was the coupling of a protected linker disaccharide derivative (**58**) with a mono-, di-, or trigalactofuranosyl thioglycoside (**17**, **54**, or **53**, respectively).

## Introduction

All mycobacteria, including the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*, possess a unique and particularly impermeable cell wall that provides the organism with significant protection from its environment.[1] Because of the role the cell wall plays in mycobacterial survival and pathogenicity, enzymes involved in its biosynthesis have been and continue to be important targets for drug action.[2] For example, among the standard antibiotics used to treat tuberculosis, two, isoniazid and ethambutol, prevent the assembly of a key structural element of the mycobacterial cell wall, the mycolyl-arabinogalactan (mAG) complex.[3]

The mAG is a large, structurally complex glycolipid that is covalently attached to cell wall peptidoglycan via a phosphate

(1) (a) Brennan, P. J. *Tuberculosis* **2003**, *83*, 91–97. (b) Lowary, T. L. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; pp 2005−2080.

(2) (a) Janin, Y. L. *Bioorg. Med. Chem.* **2007**, *15*, 2479–2513. (b) Sharma, K.; Chopra, P.; Singh, Y. *Exp. Opin. Ther. Targets* **2004**, *8*, 79–9. (c) Kremer, L.; Besra, G. S. *Exp. Opin. Inv. Drugs* **2002**, *11*, 153–157. (d) Lowary, T. L. *Mini-Rev. Med. Chem.* **2003**, *3*, 694–707.

10.1021/jo800457j CCC: $40.75 
Published on Web 05/20/2008

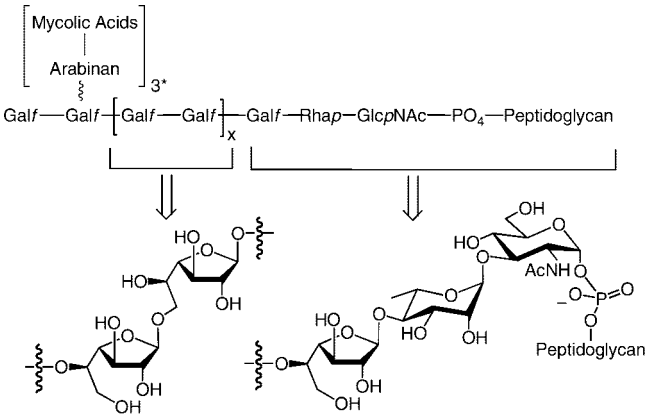



**FIGURE 1.** Schematic depiction of the mycobacterial mAG complex highlighting the structure of the galactan and linker region. $x \approx 13-15$. (*) The three arabinan chains are proposed to be attached to the 8th, 10th and 12th Gal*f* residues.[5]

linkage (Figure 1).[1,4] From this phosphate moiety extends a motif, termed the linker disaccharide, which has an α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranosyl (α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc) structure. The linker disaccharide is further elaborated, at O-4 of the Rha*p* residue, with a galactan composed of 30−35 alternating β-(1→5) and β-(1→6) linked galactofuranosyl (Gal*f*) units. Along this galactan backbone are three branch points to which are attached the arabinan domains, each consisting of, on average, 31 arabinofuranosyl residues.[5a] A recent study, in which the structure of the mAGP from *Corynebacterium glutamicum* was investigated, clearly demonstrated that the three arabinan chains are linked to O-5 of the 8th, 10th and 12th residues of the galactan backbone.[5b] Given that corynebacteria and mycobacteria both produce a similar mAGP, it has therefore been proposed[5] that a similar number of arabinan domains, attached via the same linkage points, decorate the mycobacterial galactan. The final structural element of the mAG are mycolic acids, long-chain branched lipids, which are esterified to the terminal ends of the arabinan motifs.

The biosynthesis of the mAG has been slowly unraveled over the past several years,[4,6] and while much remains to be understood, a picture has emerged about how the galactan portion of the molecule is assembled. On the basis of currently available evidence, the proposed model involves only two bifunctional galactofuranosyltransferases.[7] One, encoded in *M. tuberculosis* by the Rv3782 gene (GlfT1),[8,9] adds the first and second Gal*f* residues to the Rha*p* moiety and thus possesses both Gal*f*-β-(1→5)-Gal*f* and Gal*f*-β-(1→4)-Rha*p* transferase activity. The second enzyme (GlfT2), arising in *M. tuberculosis* from the expression of the Rv3808c gene,[10,11] has both Gal*f*-β-(1→5)-Gal*f* and Gal*f*-β-(1→6)-Gal*f* transferase activity and adds the remaining Gal*f* residues. As the donor species, both enzymes use UDP-Gal*f*, which is produced from UDP-Gal*p* by the action of UDP-Gal*p* mutase.[12]

In previous studies, we[7,11] and others[10b,c,13] have used synthetic fragments of the mAG complex to probe the specificity of these galactofuranosyltransferases. We describe here the synthesis of a panel of these compounds (Chart 1), including disaccharides **1** and **2**, trisaccharides **3** and **4**, tetrasaccharides **5** and **6**, and a series of compounds containing the linker disaccharide motif (**7**−**10**). In addition, we report the preparation of analogs of **1** and **2** in which the nonreducing Gal*f* residue has been replaced by an L-arabinofuranosyl moiety (**11** and **12**) and also a one-pot approach to trisaccharides **3** and **4**. Compounds **11** and **12** were chosen for synthesis to probe the effect that removing the terminal hydroxymethyl substituent in disaccharides **1** and **2** had on recognition by GlfT2. The work described here complements other previous investigations in which di-[13,14] and trisaccharide[15] fragments of mycobacterial galactan have been synthesized. To the best of our knowledge, no syntheses of tetragalactofuranosyl fragments of this polysaccharide (e.g., **5** and **6**) have been reported, nor have tri-, tetra- or pentasaccharide fragments containing the α-L-Rha*p*-(1→3)-α-D-Glc*p*NAc motif (e.g., **8**−**10**).

## Results and Discussion

**Synthesis of Galactofuranosyl Oligomers 1−6 and Arabinofuranosyl-Containing Disaccharides 11 and 12.** We chose to synthesize all of the targets as octyl glycosides on the basis of previous studies demonstrating that oligosaccharides contain-

(3) (a) Schroeder, E. K.; De Souza, O. N.; Santos, D. S.; Blanchard, J. S.; Basso, L. A. *Curr. Pharm. Biotechnol.* **2002**, *3*, 197–225. (b) Belanger, A. E.; Besra, G. S.; Ford, M. E.; Mikusová, K.; Belisle, J. T.; Brennan, P. J.; Inamine, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11919–11924.

(4) (a) Crick, D. C.; Mahapatra, S.; Brennan, P. J. *Glycobiology* **2001**, *11*, 107R–118R. (b) Mahapatra, S.; Basu, J.; Brennan, P. J.; Crick, D. C. Structure, Biosynthesis and Genetics of the Mycolic Acid-Arabinogalactan-Peptidoglycan Complex. In *Tuberculosis and the Tubercle Bacillus*; Cole, S. T., Eisenach, K. D., McMurray, D. N., Jacobs, W. R., Jr., Eds.; American Society for Microbiology: Washington, DC, 2005; pp 275−285.

(5) (a) Bhamidi, S.; Scherman, M. S.; Rithner, C. D.; Prenni, J. E.; Chatterjee, D.; Khoo K.-H.; McNeil, M. R. *J. Biol. Chem.* **2008**, *283*, 12992–13000. (b) Alderwick, L. J.; Radmacher, E.; Seidel, M.; Gande, R.; Hitchen, P. G.; Morris, H. R.; Dell, A.; Sahm, H.; Eggeling, L.; Besra, G. S. *J. Biol. Chem.* **2005**, *280*, 32362–32371.

(6) Berg, S.; Kaur, D.; Jackson, M.; Brennan, P. J. *Glycobiology* **2007**, *17*, 35R–56R.

(7) Beláňová, M.; Dianišková, P.; Brennan, P. J.; Completo, G. C.; Rose, N. L.; Lowary, T. L.; Mikušová, K. *J. Bacteriol.* **2008**, *190*, 1441–1445.

(8) Mikušová, K.; Beláňová, M.; Kordulakova, J.; Honda, K.; McNeil, M. R.; Mahapatra, S.; Crick, D. C.; Brennan, P. J. *J. Bacteriol.* **2006**, *188*, 6592–6598.

(9) The nomenclature for these enzymes, GlfT1 and GlfT2, was recently proposed (ref 7) and differs slightly from that used previously.

(10) (a) Mikusová, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890–33897. (b) Kremer, L.; Dover, L. G.; Morehouse, C.; Hitchen, P.; Everett, M.; Morris, H. R.; Dell, A.; Brennan, P. J.; McNeil, M. R.; Flaherty, C.; Duncan, K.; Besra, G. S. *J. Biol. Chem.* **2001**, *276*, 26430–26440. (c) Alderwick, L. J.; Dover, L. G.; Veerapen, N.; Gurcha, S. S.; Kremer, L.; Roper, D. L.; Pathak, A. K.; Reynolds, R. C.; Besra, G. S. *Protein Expression Purif.* **2008**, *58*, 332–341.

(11) Rose, N. L.; Completo, G. C.; Lin, S.-J.; McNeil, M. R.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721–6729.

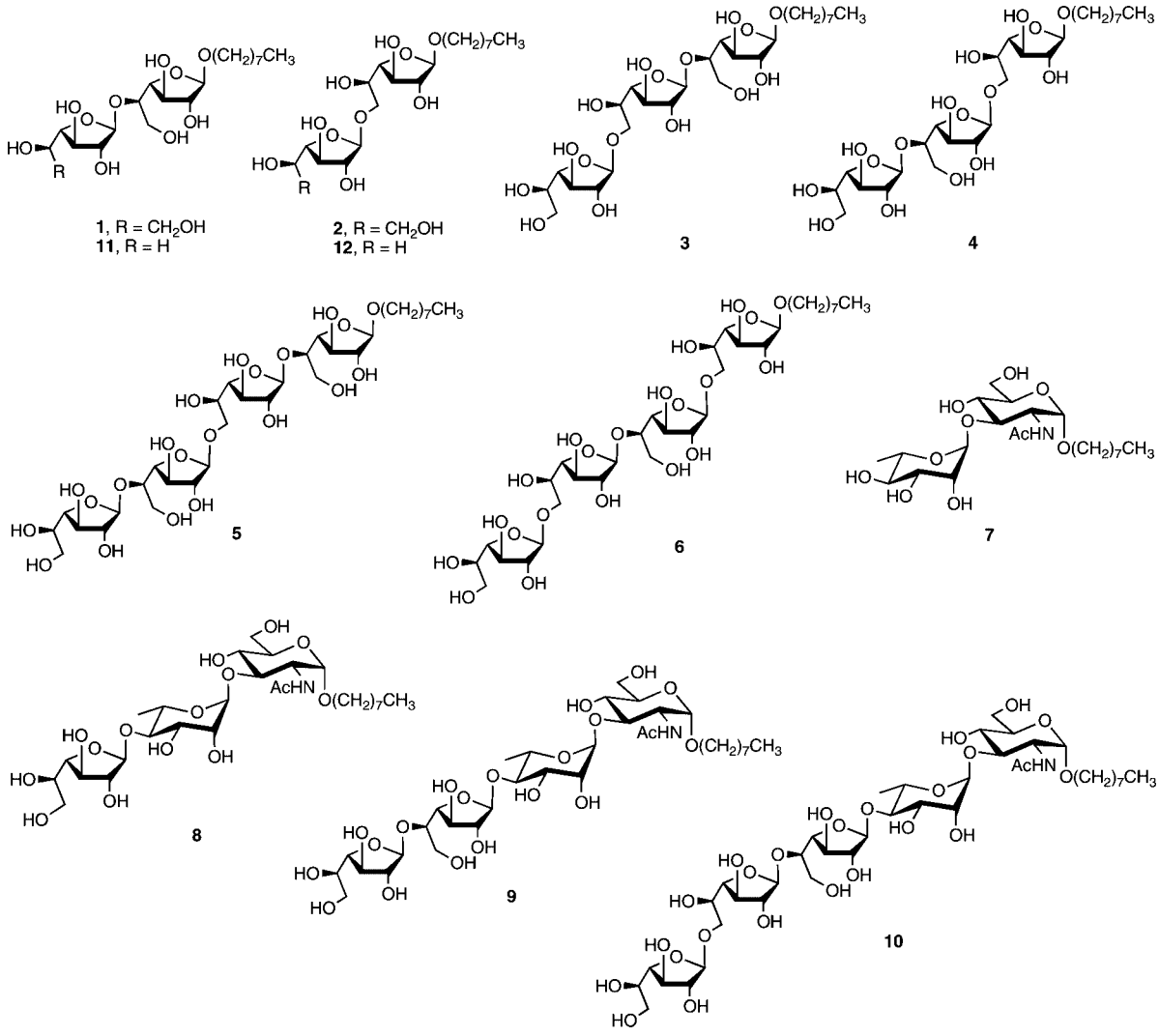
(12) Pedersen, L. L.; Turco, S. J. *Cell. Mol. Life Sci.* **2003**, *60*, 259–266.

(13) (a) Pathak, A. K.; Pathak, V.; Seitz, L.; Gurcha, S. S.; Besra, G. S.; Riordan, J. M.; Reynolds, R. C. *Bioorg. Med. Chem.* **2007**, *15*, 5629–5650. (b) Wen, X.; Crick, D. C.; Brennan, P. J.; Hultin, P. G. *Bioorg. Med. Chem.* **2003**, *11*, 3579–3587. (c) Pathak, A. K.; Besra, G. S.; Crick, D.; Maddry, J. A.; Morehouse, C. B.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **1999**, *7*, 2407–2413. (d) Pathak, A. K.; Pathak, V.; Seitz, L.; Maddry, J. A.; Gurcha, S. S.; Besra, G. S.; Suling, W. J.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *9*, 3129–3143.

(14) (a) Euzen, R.; Ferrières, V.; Plusquellec, D. *Carbohydr. Res.* **2006**, *341*, 2759–2768. (b) Sugawara, F.; Nakayama, H.; Ogawa, T. *Agric. Biol. Chem.* **1986**, *50*, 1557–156. (c) de Lederkremer, R. M.; Marino, C.; Varela, O. *Carbohydr. Res.* **1990**, *200*, 227–235. (d) McAuliffe, J. C.; Hindsgaul, O. *J. Org. Chem.* **1997**, *62*, 1234–1239. (e) Velty, R.; Benvegnu, T.; Gelin, M.; Privat, E.; Plusquellec, D. *Carbohydr. Res.* **1997**, *299*, 7–14. (f) Choudhury, A. K.; Roy, N. *Carbohydr. Res.* **1998**, *308*, 207–211. (g) Veeneman, G.; Notermans, S.; Liskamp, R. M. J.; van der Marel, G. M.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 344–350. (h) van Heeswijk, W. A. R.; Visser, H. G. J.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1977**, *59*, 81–86. (i) Pathak, A. K.; El-Kattan, Y. A.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998**, *12*, 1497–1500. (j) Marino, C.; Varela, O.; de Lederkremer, R. M. *Carbohydr. Res.* **1989**, *190*, 65–76.

(15) (a) Gandolfi-Donadío, L.; Gallo-Rodriguez, C.; de Lederkremer, R. M. *J. Org. Chem.* **2003**, *68*, 6928–6934. (b) Gandolfi-Donadío, L.; Gallo-Rodriguez, C.; de Lederkremer, R. M. *Carbohydr. Res.* **2008**, Epub ahead of print; doi: 10.1016/j.carres.2008.01.024.

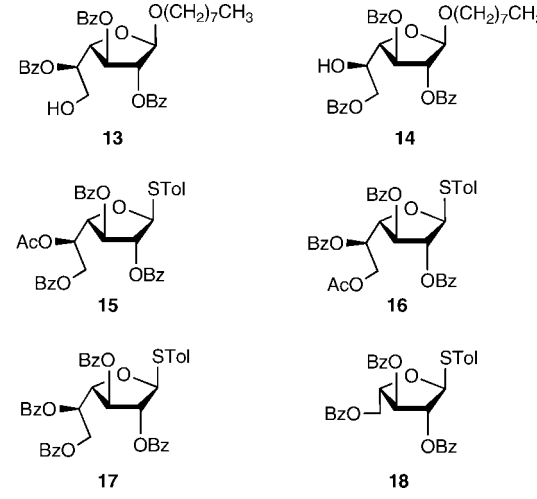
**CHART 1**




ing long-chain aglycones are better substrates for mycobacterial glycosyltransferases than glycosides of short-chain alcohols.[16] In addition, the octyl group would facilitate enzyme assays by allowing the use of reverse-phase cartridges to purify crude incubation mixtures.[17] On the basis of these considerations, we chose the known[13d] octyl glycosides **13** and **14** (Chart 2) as the building blocks for the "reducing" end of the targets. We envisioned that thioglycosides **15**−**18** could be used to introduce the other residues via an iterative process involving glycosylation and selective deacetylation reactions.

In developing a route to **13**−**18**, the first issue to address was to identify a method for efficiently obtaining a galactose intermediate in the furanose ring form. There are a variety of processes reported for carrying out this transformation, with the most common being Fischer glycosylation,[18] which when carried out under kinetic control affords the higher energy furanoside isomers. However, in our hands, the use of this approach led to mixtures of the furanosides contaminated with the more thermodynamically stable pyranosides, which were difficult to separate. Other methods for the preparation of galactofuranoside derivatives include the reduction of galactonolactones,[19] the

**CHART 2**



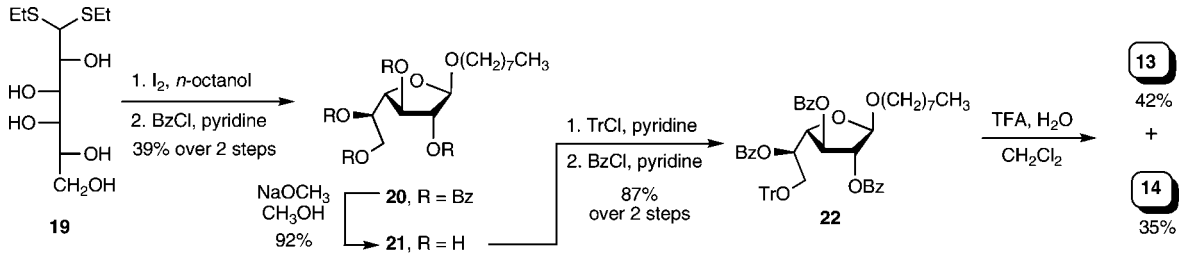

(16) Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121–1128.

(17) Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 59–63.

(18) (a) Arasappan, A.; Fraser-Reid, B. *Tetrahedron Lett.* **1995**, *36*, 7967–7070. (b) Ferrières, V.; Bertho, J.-N.; Plusquellec, D. *Carbohydr. Res.* **1998**, *311*, 25–35.

(19) Kohn, P.; Samaritano, R. H.; Lerner, L. M. *J. Am. Chem. Soc.* **1965**, *87*, 5469–5472.

**SCHEME 1**




acid-catalyzed ring opening of 1,4-anhydrogalactopyranose derivatives,[20] the high-temperature benzoylation of galactose,[21] anomeric alkylation,[22] and the cyclization of open-chain *S*,*O*-acetals[14d] or dithioacetals[23] with mercuric salts. A recent paper describes Fischer glycosylation of D-galacturonic acid in methanol followed by the reduction of the product methyl glycoside methyl ester, which affords good yields of galactofuranosides over the two-step process.[24]

Previously,[25] we explored a recently described variation of the dithioacetal cyclization approach, in which galactose dithioacetals were cyclized with 1,3-dibromo-5,5-dimethylhydantoin.[26] Although this reaction afforded the desired product, in our hands the yield was modest and we therefore turned to an alternate method, reported by Szarek and co-workers,[27] which involves treatment of the dithioacetal with an alcohol and iodine. An attractive feature of the Szarek method is that the promoter is inexpensive and relatively benign. The method was reported to be highly selective for producing furanosides from dithioacetals of glucose, arabinose and mannose; curiously, it has not, to the best of our knowledge, been applied to the synthesis of galactofuranose derivatives. As detailed below, we have found that the iodine-promoted cyclization of galactose diethyl dithioacetal does indeed efficiently provide galactofuranosides inexpensively and free from contamination by galactopyranosides.

**Preparation of Octyl Glycoside Acceptors.** The synthesis of **13** and **14** (Scheme 1) proceeded from dithioacetal **19**[28] by reaction with iodine (5%, by weight) in *n*-octanol. Although the original report with diothioacetals derived from other monosaccharides used only 0.5% iodine when methanol was used as the solvent,[27] in *n*-octanol the reaction was very sluggish at this concentration and hence a larger amount of the promoter was used. Next, the crude product obtained after workup and removal of the *n*-octanol was suspended in pyridine before benzoyl chloride was added. While benzoylation was not essential, doing so facilitated isolation of the product, **20**, which was obtained as a 9:1 $\beta$:$\alpha$ mixture. The $\beta$-glycoside was successfully purified by chromatography and subsequent treatment with sodium methoxide thus afforded **21** in 36% yield in three steps from **19**. The $\beta$-stereochemistry of the major product could be readily established by NMR spectroscopy. In the $^1$H NMR spectrum of **21**, the anomeric hydrogen resonance appeared as a singlet at 5.24 ppm, and in the $^{13}$C NMR spectrum the anomeric carbon resonance appeared at 105.7 ppm. Both are consistent with the $\beta$-galactofuranoside stereochemistry.[29] We note that the stereoselectivity observed in this glycosylation reaction is of the same magnitude as that reported previously in cyclization of acyclic *S*,*O*-acetals.[14d]

With **21** in hand, reaction with trityl chloride in pyridine followed by benzoyl chloride afforded the fully protected derivative **22** in 87% overall yield over the two steps. The required acceptor alcohols **13** and **14** were obtained upon treatment of **22** with trifluoroacetic acid in wet dichloromethane, as reported previously.[13d] Under these conditions, primary alcohol **13** was obtained in 42% yield, while the secondary alcohol **14**, resulting from acid catalyzed migration of the O-5 benzoate ester, was isolated in 35% yield.

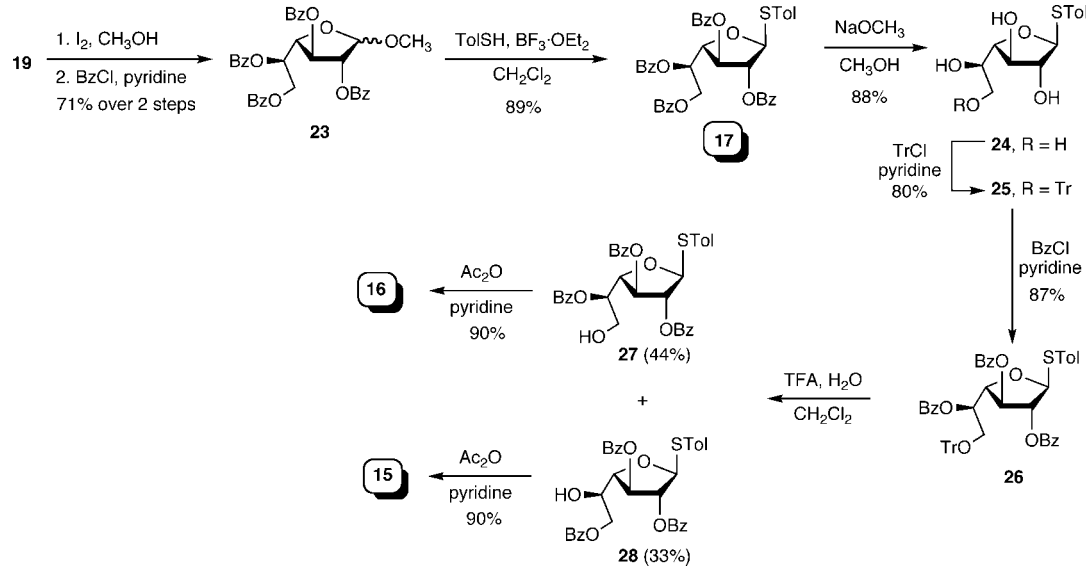
**Preparation of Thioglycoside Donors.** An analogous approach was used to synthesize thioglycosides **15**−**17** (Scheme 2). Dithioacetal **19** was converted into methyl glycoside **23** in two steps and 71% yield upon reaction with iodine (2% by weight) in methanol[30] followed by benzoylation. As was the case for the synthesis of **21**, the glycosylation proceeded with high $\beta$-selectivity ($\beta$:$\alpha$ ratio of 9:1). Methyl glycoside **23** was next converted to the corresponding thioglycoside **17** upon reaction with *p*-thiocresol and boron trifluoride etherate, which afforded an 89% yield of the product. Transformation of **17** to the remaining two galactofuranosyl thioglycoside donors, **15** and **16**, was straightforward. Removal of the benzoate esters of **17** yielded the deprotected thioglycoside **24** (88%), which was then tritylated (yielding **25**) and benzoylated to give **26** in 70% yield over the two steps. Subsequent treatment of **26** with trifluoroacetic acid as described for the preparation of **13** and **14**, yielded the expected thioglycoside alcohols **27** and **28** in 44% and 33% yields, respectively. The structures of **27** and **28** could be readily differentiated by NMR spectroscopy of the products. In **27**, the signal for the H-5 resonance is significantly downfield (5.69 ppm) as would be expected as O-5 is acylated. In contrast, in the NMR spectrum for **28**, H-5 appears at 4.59 ppm. Reaction of **27** with acetic anhydride and pyridine proceeded uneventfully affording a 90% yield of **16**; similar treatment of **28** yielded **15**, also in 90% yield.

The remaining thioglycoside, **18**, was synthesized from the known[31] methyl glycoside **29** in 89% yield upon treatment with *p*-thiocresol and boron trifluoride etherate (Scheme 3).

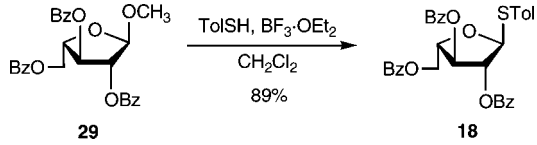
**Synthesis of Disaccharides 1, 2, 11 and 12.** Disaccharides **1** and **2** were synthesized by coupling either octyl glycoside **13** or **14** with thioglycoside **17** followed by deprotection of the

(20) Kovensky, J.; Sinaÿ, P. *Eur. J. Org. Chem.* **2000**, 3523–2525.
(21) D'Accorso, N. B.; Thiel, I. M. E.; Schüller, M. *Carbohydr. Res.* **1983**, *124*, 177–184.
(22) Gola, G.; Libenson, P.; Gandolfi-Donadío, L.; Gallo-Rodriguez, C. *ARKIVOC* **2005**, *xii*, 234–242.
(23) Green, J. W.; Pacsu, E. *J. Am. Chem. Soc.* **1938**, *60*, 2056–2057.
(24) Bordoni, A.; de Lederkremer, R. M.; Marino, C. *Tetrahedron* **2008**, *64*, 1703–1710.
(25) Bai, Y.; Lowary, T. L. *J. Org. Chem.* **2006**, *71*, 9658–9671.
(26) Madhusudan, S. K.; Misra, A. K. *Carbohydr. Res.* **2005**, *340*, 497–502.
(27) Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. *Tetrahedron Lett.* **1986**, *27*, 3827–3830.
(28) Norris, P.; Horton, D. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 35−52.
(29) Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504–2511.
(30) The increased amount of iodine, compared to that originally reported (ref 27) was to speed the completion of the reaction.
(31) Hatfield, R. D.; Helm, R. F.; Ralph, J. *Anal. Biochem.* **1991**, *194*, 25–33.

**SCHEME 2**




**SCHEME 3**




resulting product as illustrated in Scheme 4. Thus, reaction of **14** with **17** in the presence of *N*-iodosuccinimide (NIS) and silver triflate (AgOTf)[32] afforded disaccharide **30** in 87% yield. For this and the remainder of the glycosylation products described in this paper, the anomeric stereochemistry of the newly introduced Gal*f* residue was determined by NMR spectroscopy from the magnitude of the $^3J_{H\text{-}1,H\text{-}2}$ and the chemical shift of the resonance for the anomeric carbon. In all cases, the $^3J_{H\text{-}1,H\text{-}2}$ magnitude was between 0 and 2 Hz, while the chemical shift of the anomeric carbon was between 104.0 and 106.8 ppm (107.9−110.6 ppm after full deprotection). These $^{13}$C and $^1$H NMR data both support the $\beta$-galactofuranoside stereochemistry.[29] Deprotection of **30** was achieved upon treatment with sodium methoxide, which provided **1** in 90% yield. Disaccharide **2** was synthesized via an identical series of transformations: glycosylation of **13** with **17** gave disaccharide **31** in 89% yield, which was then deprotected affording **2** (87%).

The same general approach was also used to prepare the L-arabinofuranosyl-containing disaccharides, **11** and **12** (Scheme 5). First, reaction of alcohol **14** with thioglycoside **18** upon activation with NIS–AgOTf afforded disaccharide **32** (87% yield), which was then deprotected giving **11** in 90% yield. Application of the same transformations to **13** yielded **12** by way of **33** in 82% yield overall yield.

**Synthesis of Trisaccharides 3 and 4.** The preparation of trisaccharides **3** and **4** also began from octyl glycosides **13** and **14** as illustrated in Scheme 6. Reaction of **14** and **16** promoted by NIS and AgOTf afforded the differentially protected disaccharide **34** in 89% yield. The acetyl group was selectively cleaved upon treatment with HCl in methanol,[33] which provided, in 75% yield, alcohol **35**. Under these conditions an isomeric product, resulting from migration of the benzoyl group from O-5 to O-6 (**36**, Chart 3), was also produced in 20% yield. Given the efficiency of the TFA-promoted acyl migration during the deprotection of **22** and **26**,[13d] which was exploited in the preparation of **13**−**16** (above), the formation of byproduct **36** under these acidic deacetylation conditions is perhaps not unexpected. Regardless, isomers **35** and **36** could be easily separated by chromatography and the latter could be deprotected to afford **1**. Subsequent reaction of **35** with **17** gave trisaccharide **37**, which was then debenzoylated affording **3** in 70% yield over the two steps.

In a similar manner, trisaccharide **4** was synthesized by first coupling **13** with **15** to give disaccharide **38** in 99% yield. Selective deacetylation under acidic conditions provided **39** in 85% yield together with 10% of a compound believed to be the benzoyl migration product, **40** (Chart 3). The lower amount of benzoyl migration observed upon deacetylation of **38** as opposed to **34** is presumably due to the tendency of acyl groups to migrate to primary positions.[34]Because the product formed initially from **36** has a benzoate ester at the primary position, there is consequently little driving force for acyl migration. Glycosylation of **39** with **17** followed by deprotection with sodium methoxide afforded, over the two steps, an 81% yield of target **4**.

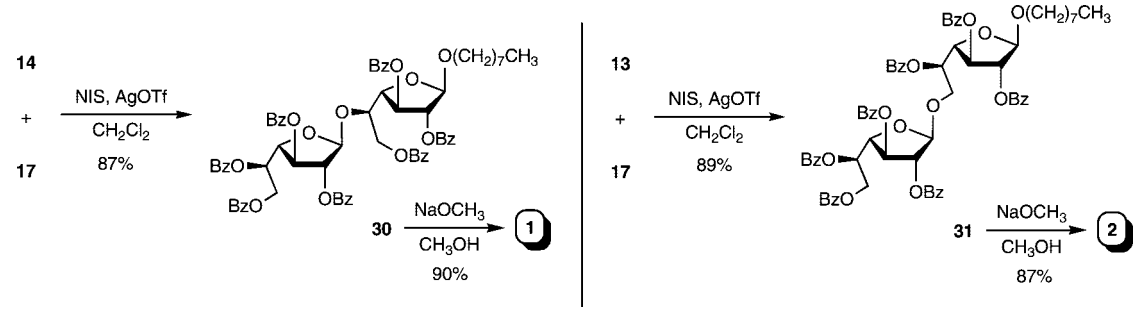
**Synthesis of Tetrasaccharides 5 and 6.** As illustrated in Scheme 7, the synthesis of tetrasaccharide **5** was achieved from disaccharide **35** by application of the same iterative approach described above for the preparation of trisaccharides **3** and **4**. Thus, glycosylation of **35** with thioglycoside **15** proceeded to give trisaccharide **42** in 78% yield. Deacetylation with acidic methanol gave **43** in 86% yield together with 8% of a product presumed to be the benzoyl migrated species, **44** (Chart 3), which were separated by chromatography. The final monosaccharide residue was introduced by the coupling of **43** with **17**, which afforded a 70% yield of tetrasaccharide **45**. Deprotection of **45** with sodium methoxide yielded **5** in 82% yield.

(32) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.

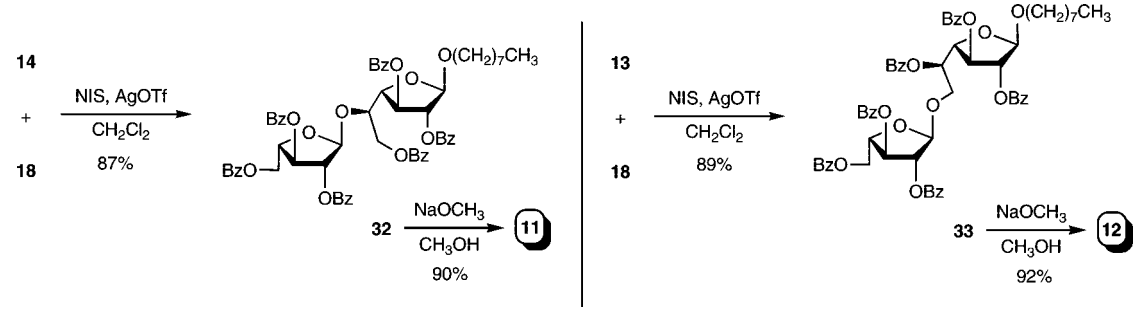
(33) Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8−C11.

(34) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11–109.

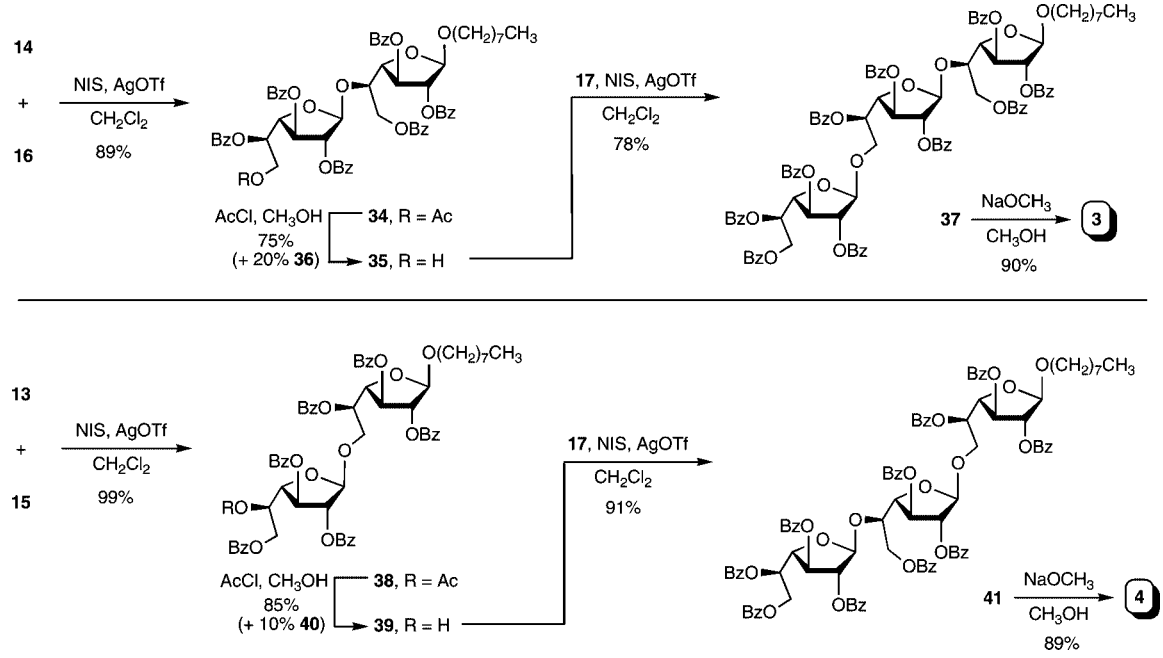
**SCHEME 4**




**SCHEME 5**




**SCHEME 6**

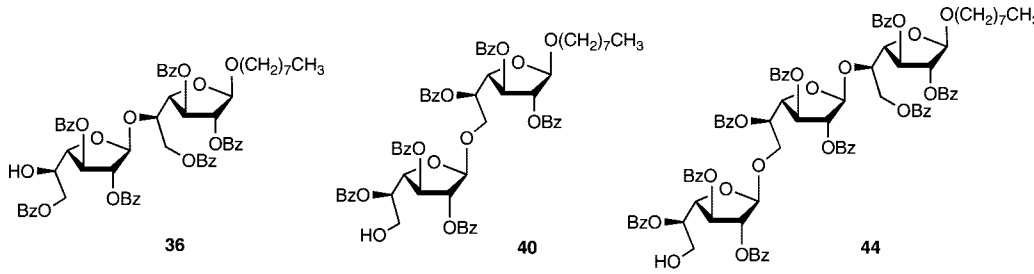



In contrast to the synthesis of the other oligosaccharides, the preparation of **6** proved to be more problematic. Following the same general approach used for the synthesis of **5**, the coupling of **39** with **16** initially produced the corresponding trisaccharide **46** together with the hydrolyzed donor, which coeluted during purification by chromatography. This mixture of compounds was then subjected to selective acetyl group deprotection to afford pure alcohol **47** in 42% yield over two steps. The corresponding acyl migration product (8%) was also observed as a side product. However, with **47** in hand, all our attempts to further glycosylate it with **17** failed to give any tetrasaccharide product. A variety of reaction conditions were explored in an attempt to produce the desired target from **47** and **17**, but to no avail. We also investigated if trichloroacetimidate **49** (Chart 4)[35] could serve as an effective donor for the preparation of **48** but were unsuccessful, under a number of conditions. We do not know the reasons why this stepwise approach, which had been effective for the synthesis of **5**, failed to produce **6**. One possibility is that trisaccharide alcohol **47** adopts a conformation in which the reactive alcohol is somehow hindered, which in turn substantially reduces its nucelophilicity.

Faced with this problem, we explored an alternative strategy to **48**, in which the molecule would be built from the nonre-

(35) Gallo-Rodriguez, C.; Gandolfi, L.; de Lederkremer, R. M. *Org. Lett.* **1999**, *1*, 245–247.

**CHART 3**




**SCHEME 7**

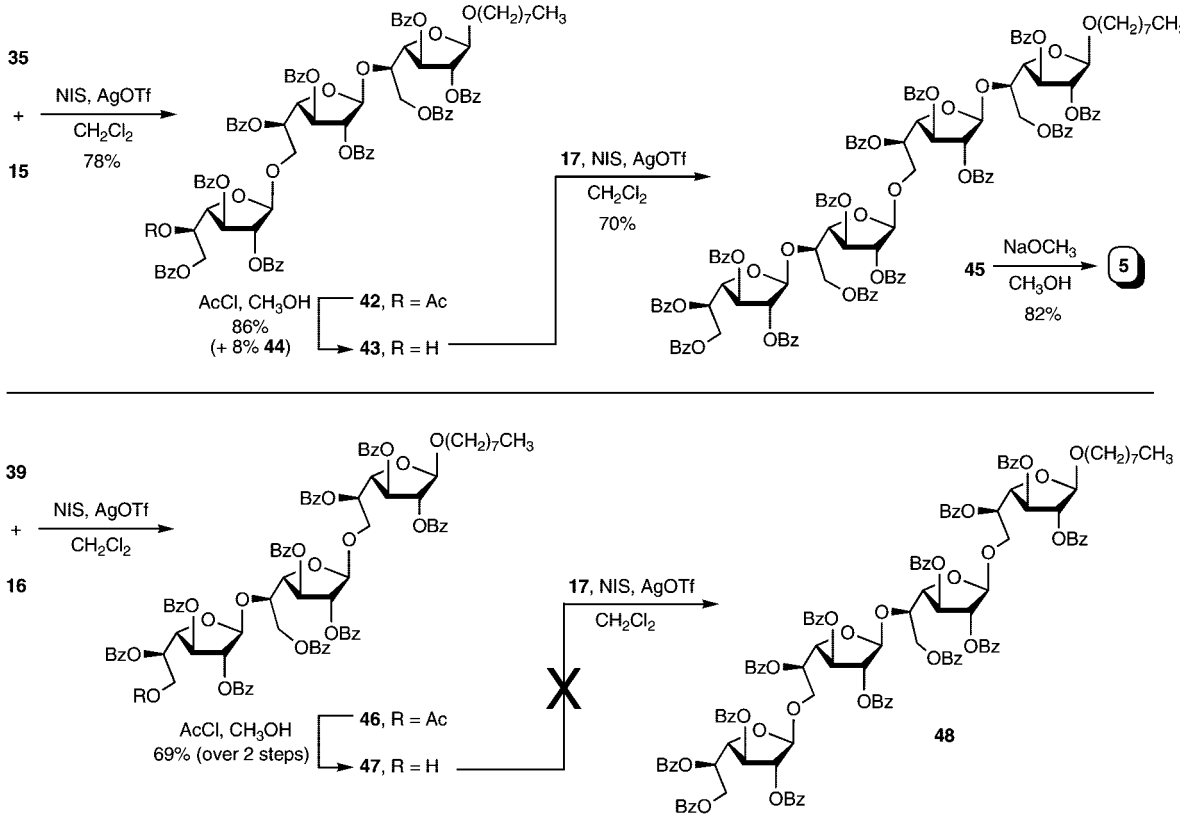
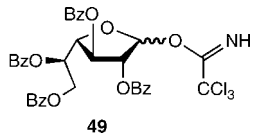


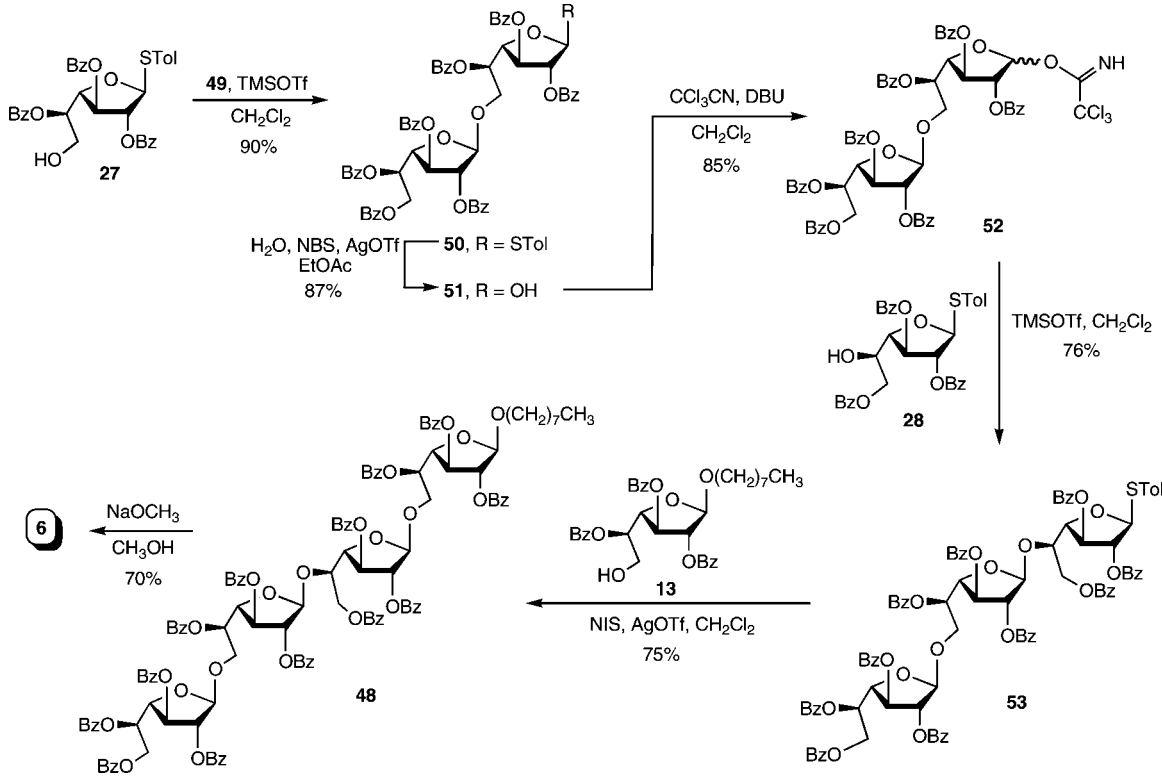

**CHART 4**



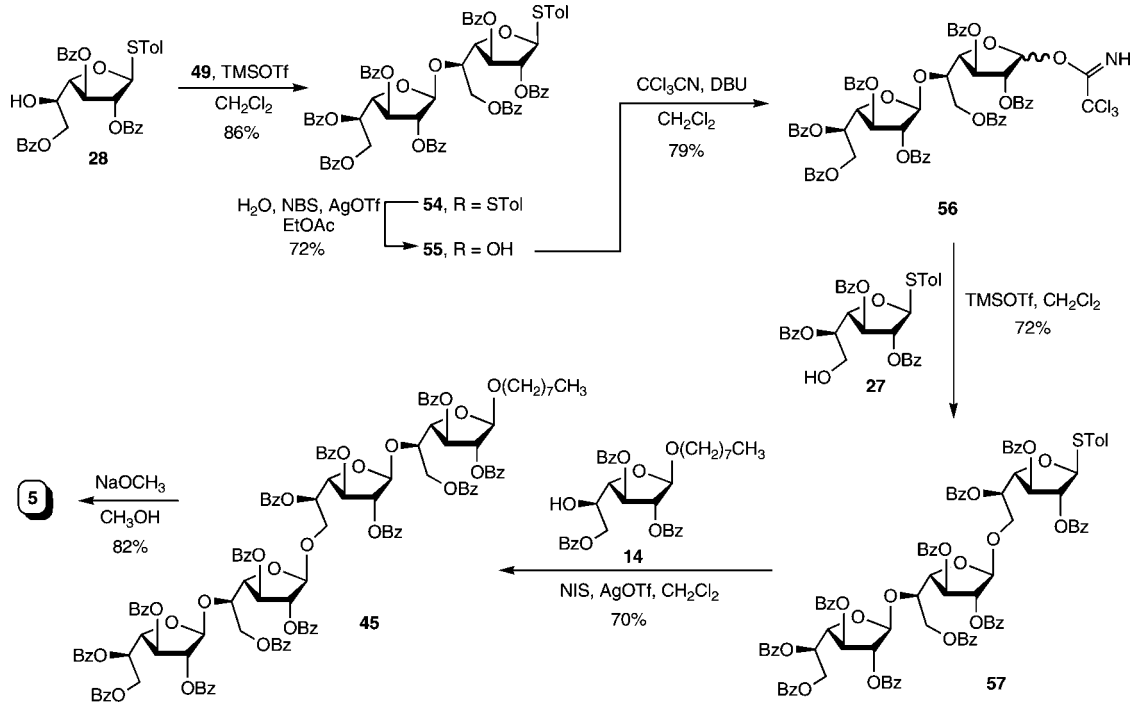

ducing end to the reducing end. This approach, shown in Scheme 8, was successful and involved the use of thioglycoside alcohols **27** and **28**, the synthesis of which was described earlier (see Scheme 2). Thus, thioglycoside alcohol **27** was glycosylated with trichloroacetimidate **49** promoted by TMSOTf, which gave disaccharide **50**, in 90% yield. This thioglycoside alcohol was then hydrolyzed to produce an 87% yield of hemiacetal **51**, which was then converted to the corresponding trichloroacetimidate **52** in 85% yield. With this disaccharide donor in hand, it was used to glycosylate alcohol **28**, thus affording trisaccharide thioglycoside **53** in 76% yield. Finally, this thioglycoside was activated using NIS–AgOTf in the presence of octyl glycoside acceptor **13**, giving the desired tetrasaccharide **48** in 75% yield. Treatment with sodium methoxide then afforded a 70% yield of **6**.

An obvious advantage of this alternate strategy was that it allowed us to access **6** successfully. Less obvious but still important is that by building up the molecule from the nonreducing to reducing end, it was possible to avoid the need for selective deacetylation reactions at either O-5 or O-6, which in all cases led to migration of benzoyl groups to the adjacent positions (see Schemes 6 and 7). Therefore, we also explored this approach to synthesize tetrasaccharide **5**, and this was also successful. As illustrated in Scheme 9, starting from imidate **49** and thioglycoside alcohol **28**, the thioglycoside disaccharide **54** was obtained in 86% yield. Hydrolysis of the thioglycoside by NBS in wet ethyl acetate gave **55** (72% yield), and in turn, imidate derivative **56** was obtained in 79% yield upon reaction with trichloroacetonitrile and DBU. TMSOTf-promoted glycosylation of alcohol **27** with this imidate yielded an 72% yield of trisaccharide **57**. The synthesis of **5** was then completed by reaction of **57** with **14** using NIS–AgOTf as the promoter, leading to a 70% yield of the fully protected tetrasaccharide **45**, which was then deacylated using sodium methoxide in methanol yielding **5** in 82% yield. Overall, this route (Scheme 9) gave

**SCHEME 8**



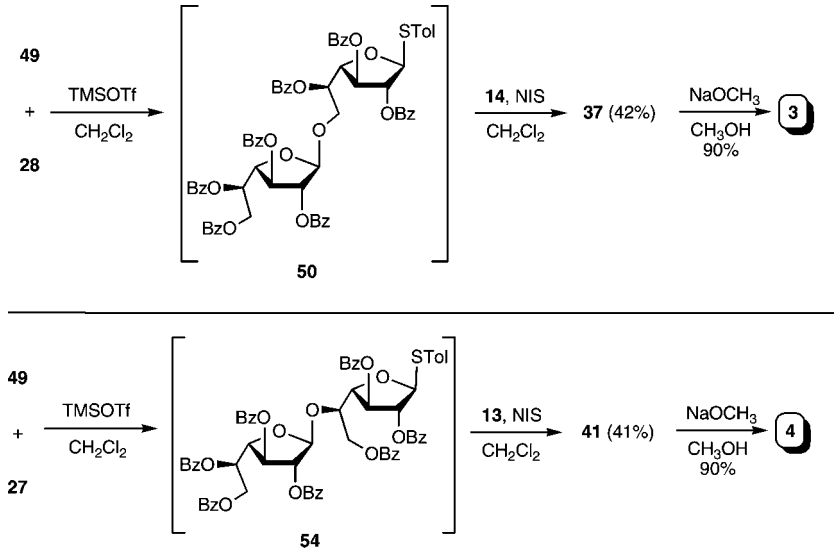

**SCHEME 9**




tetrasaccharide **5** in six steps and 20% yield from monosaccharide building blocks, compared to six steps and 26% yield for the approach shown in Schemes 6 and 7. While the first approach proved slightly better in providing tetrasaccharide **5**, the "non-reducing to reducing end" strategy may in some cases have advantages in the synthesis of large galactofuranosides. This strategy relies on glycosylation reactions with monosaccharide acceptors, which may be less prone to conformational effects that reduce the nucleophilicity of the acceptor alcohol. As the size of the chain increases such conformational effects might be expected to increase.

**One-Pot Syntheses of Trisaccharides 37 and 41.** Our earlier studies[11] on one of the mycobacterial galactofuranosyltranserases, GlfT2, revealed that trisaccharides **3** and **4** were optimal substrates for this enzyme. In anticipation of needing to screen a substantial number of inhibitors of the enzyme using a recently

**SCHEME 10**




developed high-throughput assay for the enzyme,[36] it was necessary to have in place a robust and efficient route for the preparation of a large amount of these compounds, which would be needed in every inhibitor assay. Although the route illustrated in Scheme 6 provided both targets in good overall yield, the benzoyl migration that occurred upon deacetylation of disaccharide intermediates **34** and **38** under acidic conditions was a limitation of this approach. While this could, in theory, be solved by replacing the acetyl fuctionality with another group (e.g., levulinoyl), which could be taken off under conditions to which the benzoyl groups would be stable (hydrazine acetate or sodium borohydride), we nevertheless were concerned that even under these conditions some acyl migration would occur.[34]

Given the success we had in synthesizing tetrasaccharides **5** and **6** via the approach outlined in Schemes 8 and 9, in which the molecules were assembled from the nonreducing to reducing end, we viewed this as an attractive strategy for the efficient synthesis of **3** and **4**. In addition to eliminating the need for selective deprotection reactions, we envisioned that these reactions could be carried out in one-pot[37] from trichloroacetimidate **49**, thioglycosides **27** or **28**, and octyl glycosides **13** or **14**, thus dramatically speeding the production of these compounds. The one-pot method is made feasible as only two glycosylation reactions are needed for the synthesis of **3** and **4**, and we had in hand two classes of donors, thioglycosides and trichloroacetimidates, the latter of which could be selectively activated in the presence of the former.

The successful implementation of this approach is shown in Scheme 10. Thus trisaccharide **37** (and in turn **3**) could be obtained by reacting trichloroacetimidate **49** with thioglycoside alcohol **28** under the promotion of TMSOTf in $CH_2Cl_2$ at room temperature. After 30−60 min, a new spot, disaccharide **50**,[38] was visible on TLC. Addition of octyl glycoside alcohol **14**, NIS and AgOTf led, after 15 min, to the formation of **37**. Following chromatography, **37** was obtained in 42% overall yield from **28**, which corresponds to an approximately 65% yield in both glycosylation steps. Using a similar series of reactions, trichloroacetimidate **49**, thioglycoside alcohol **27** and octyl glycoside **13** could be converted to thioglycoside **41**, via disaccharide **54**, in 41% overall yield (Scheme 10). Trisaccharides **37** and **41** obtained from these one-pot procedures were deacylated affording **3** and **4** in 90% and 89% yields, respectively. This route, in which the sugars are added from the nonreducing end to the reducing end of the molecule has clear advantages over the alternate approach we investigated earlier (Scheme 6). Not only can the product be obtained rapidly (within 1 day) in a one-pot manner, but also the acyl migration observed in the selective deacylation reactions (i.e., **38** → **39** + **40**, Scheme 6) is avoided.

**Synthesis of Oligosaccharides Containing the Linker Disaccharide (7−10).** For the preparation of the oligosaccharide targets with the linker disaccharide, **7**−**10**, a convergent strategy was adopted where an acceptor containing a suitably protected α-Rha*p*-(1→3)-α-Glc*p*NAc moiety would be coupled with an appropriate galactofuranose-containing thioglycoside, either a mono-, di-, or trisaccharide. We therefore envisioned that the step in which these two groups were joined would require disaccharide **58** and either **17**, **53**, or **54** (Figure 2). The preparation of thioglycosides **17**, **53**, and **54** was presented in Schemes 2, 8, and 9, respectively. The synthesis of octyl glycoside **58** is shown in Scheme 11.

The route to **58** started from the known benzylidene protected *N*-acetyl-glucosamine derivative **59**[39] (Scheme 11), which was glycosylated with the previously reported rhamnose thioglycoside **60**.[40] This reaction provided an 89% yield of the expected

(36) Rose, N. L.; Zheng, R. B.; Pearcey, J.; Zhou, R.; Completo, G. C.; Lowary, T. L. *Carbohydr. Res.* **2008**, Accepted for publication; doi:10.1016/j.carres.2008.03.023.

(37) (a) For some examples of one-pot approaches to oligosaccharides, see: Huang, L. J.; Wang, Z.; Li, X. N.; Ye, X. S.; Huang, X. F. *Carbohydr. Res.* **2006**, *341*, 1669–1679. (b) Yu, B.; Yang, Z. Y.; Cao, H. Z. *Curr. Org. Chem.* **2005**, *9*, 179–194. (c) Huang, X. F.; Huang, L. J.; Wang, H. S.; Ye, X. S. *Angew. Chem., Int. Ed.* **2004**, *43*, 5221–5224. (d) Codee, J. D. C.; van den Bos, L. J.; Litjens, R. E. J. N.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Org. Lett.* **2003**, *5*, 1947–1950. (e) Zhang, Z. Y.; Ollmann, I. R.; Ye, X. S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753. (f) Grice, P.; Ley, S. V.; Pietruszka, J.; Priepke, H. W. M.; Walther, E. P. E. *Synlett* **1995**, 781–784.

(38) The identity of the intermediates formed in these one-pot reactions was confirmed by TLC comparison with authentic standards of **50** and **54**, which were obtained in the course of the synthesis of **5** and **6** (Schemes 8 and 9).

(39) Aguilera, B.; Romero-Ramírez, L.; Abad-Rodríguez, J.; Corrales, G.; Nieto-Sampedro, M.; Fernández-Mayoralas, A. *J. Med. Chem.* **1998**, *41*, 4599–4606.

(40) Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863.

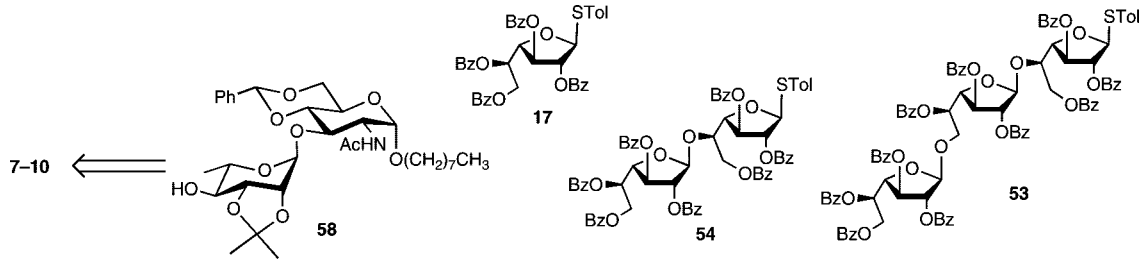
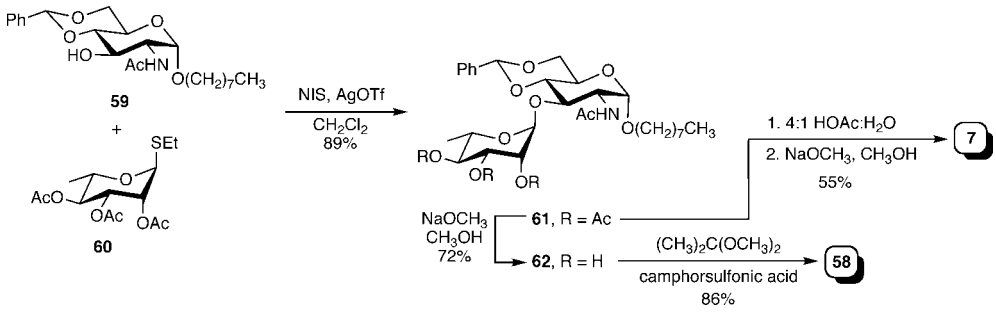



**FIGURE 2.** Retrosynthetic analysis of **7**−**10**.

**SCHEME 11**




disaccharide, **61**. The stereochemistry of the rhamnopyranosyl moiety in **61** was proven by measurement of the $^{1}J_{C\text{-}1,H\text{-}1}$ for this residue, which revealed a value of 170 Hz, consistent with the proposed α-stereochemistry.[41] A portion of **61** was treated with aqueous acetic acid at 70 °C and then with sodium methoxide thus giving disaccharide **7** in 55% yield. The remainder of **61** was deacetylated upon treatment with sodium methoxide to give triol **62** in 72% yield. Subsequent treatment of **62** with dimethoxypropane and camphorsulfonic acid afforded **58** in 86% yield.

With all the building blocks in hand, the preparation of **8**−**10** was straightforward as shown in Scheme 12. Thus, glycosylation of **58** with **17**, **54**, or **53** gave the expected oligosaccharide products **63**−**65** in 75−85% yield. Each of these compounds was then deprotected in two steps: removal of the acetal protecting groups under acidic conditions followed by debenzoylation under basic conditions. These reactions afforded **8**−**10** in 62−89% yield.

## Conclusions

In conclusion, we describe here the synthesis of a panel of oligosaccharides (**1**−**12**) that have found application as substrates for galactofuranosyltransferases involved in the biosynthesis of mycobacterial AG.[7,11] An important element of this work was the use of an iodine-promoted cyclization[27] of galactose diethyl dithioacetal (**19**) for the preparation of Gal*f* derivatives (e.g., **13**−**17**), which were required building blocks for all of the target molecules, except **7**. The major advantages of this approach for accessing the thermodynamically least stable ring form of this sugar include the use of inexpensive reagents and the preparation of furanosides in the apparent total absence of pyranosides.

With these building blocks in hand, the preparation of the disaccharides proceeded without difficulty. In the synthesis of the tri- and tetrasaccharides **3**−**6**, our initial strategy involved building up the molecules from the reducing to nonreducing end. While this allowed us to obtain trisaccharides **3** and **4**, as well as tetrasaccharide **5**, we were unable to prepare tetrasaccharide **6** by this route, which may arise from conformational features of the acceptor substrate that hinder the reactive alcohol nucleophile in the final glycosylation step. A strategy in which these compounds were assembled from the nonreducing to reducing end was then investigated and was successfully implemented for the synthesis of both tetrasaccharides **5** and **6**. In addition to allowing the preparation of these target molecules, this approach also allowed us to eliminate the need for selective deprotection of an acetyl group on O-5 or O-6 of the terminal Gal*f* residue in the oligosaccharide intermediates. This was a key feature of our initial strategy, which in all cases led to some O-5 → O-6 (or O-6 → O-5) benzoyl group migration, thus reducing yields and complicating purifications. Furthermore, this strategy enabled a one-pot synthesis of trisaccharides **3** and **4**. Finally, four oligosaccharides (**7**−**10**) that contain the linker disaccharide region of the AG and up to three Gal*f* residues were synthesized. The work described here represents the first preparation of three of these mAG fragments (**8**−**10**), which were obtained by the coupling of a key α-Rha*p*-(1→3)-α-Glc*p*NAc disaccharide intermediate with a donor containing one, two or three Gal*f* residues (**17**, **54** or **53**, respectively). Oligosaccharides **1**−**12** have already found significant application in probing mycobacterial AG biosynthesis[7,11] and further studies with these glycans are ongoing.

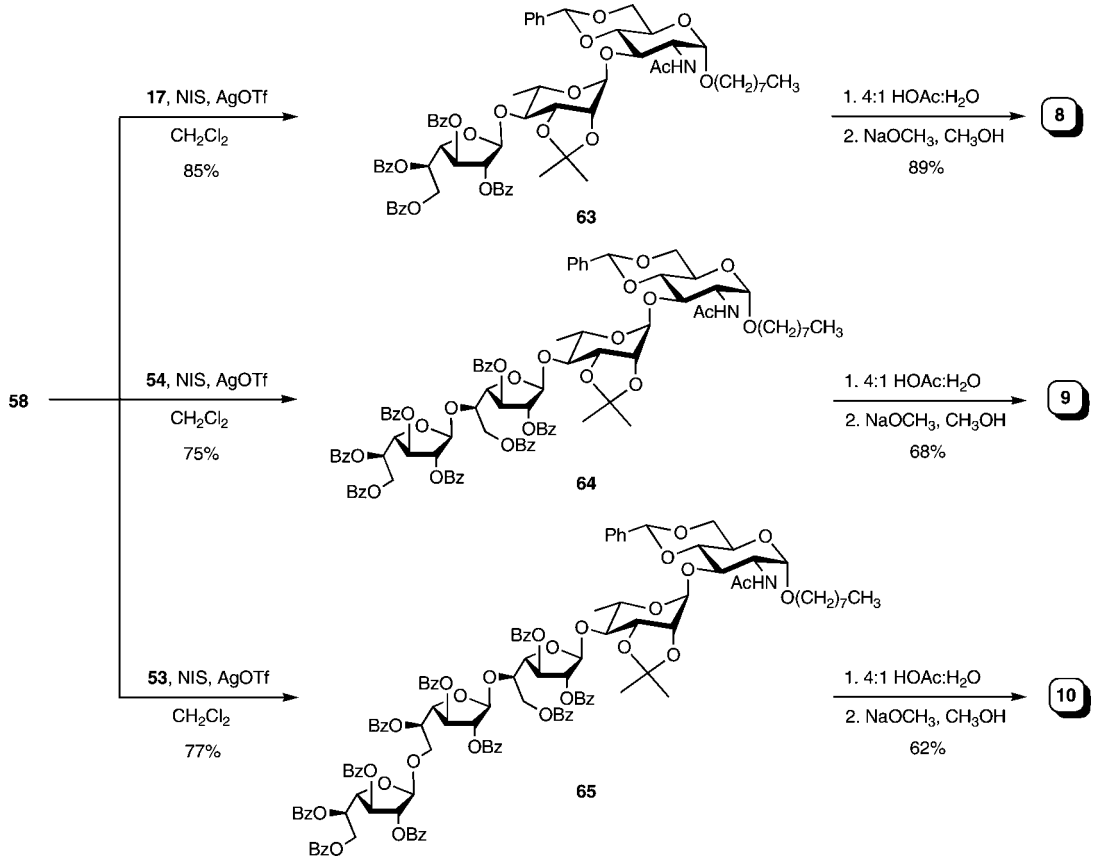
## Experimental Section

**General Procedure for Deprotection of Intermediates Leading to 1−6, 11 and 12.** The starting material was dissolved in 3:1 MeOH−$CH_2Cl_2$ (10−50 mL) followed by dropwise addition of NaOMe in MeOH (0.1 M) until the pH of the solution was 12. The reaction mixture was stirred for 4 h and was neutralized by the careful addition of Amberlyst-15 ($H^+$) cation exchange resin. The solution was filtered and the filtrate concentrated to give a syrupy residue, which was then purified by chromatography. The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge.

**General Procedure for Deprotection of Intermediates Leading to 7−10.** The starting material was dissolved in 4:1 HOAc−$H_2O$ (10−30 mL) and heated at 70 °C for 4 h. The solvent was

(41) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.

**SCHEME 12**




evaporated upon completion of the reaction. The residue was dissolved in MeOH (10−50 mL) followed by dropwise addition of NaOMe in MeOH (0.1 M) until the pH of the solution was 12. The reaction mixture was then stirred at room temperature for 4 h and was neutralized by the addition of Amberlyst-15 ($H^+$) cation exchange resin. The solution was filtered and the filtrate concentrated to give a syrupy residue. The resulting crude product was then purified by chromatography. The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge.

**Octyl *β*-D-Galactofuranosyl-(1→5)-*β*-D-galactofuranoside (1).** Using the general deprotection procedure, octyl glycoside **30** (92.0 mg, 0.078 mmol) gave a crude product which was purified by column chromatography (5:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **1** as a clear, colorless oil (32.0 mg, 90%). The data for this compound matched that previously reported.[13d]

**Octyl *β*-D-Galactofuranosyl-(1→6)-*β*-D-galactofuranoside (2).** Using the general deprotection procedure, octyl glycoside **31** (37.1 mg, 0.032 mmol) gave a crude product, which was purified by column chromatography (5:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **2** as a colorless oil (12.5 mg, 87%). The data for this compound matched that previously reported.[13d]

**Octyl *β*-D-Galactofuranosyl-(1→6)-*β*-D-galactofuranosyl-(1→5)-*β*-D-galactofuranoside (3).** Using the general deprotection procedure, octyl glycoside **37** (45.4 mg, 0.027 mmol) gave a crude product, which was purified by column chromatography (3:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish trisaccharide **3** as a clear, colorless oil (15.2 mg, 90%). $R_f$ 0.21 (3:1 $CH_2Cl_2$−MeOH); $[\alpha]_D$ −93.0 (*c* 1.0, MeOH); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$): 5.23 (d, 1 H, $J$ = 1.3 Hz, H-1′), 5.04 (d, 1 H, $J$ = 1.5 Hz, H-1″), 4.98 (d, 1 H, $J$ = 2.0 Hz, H-1), 4.14−3.94 (m, 9 H, H-2″, H-2′, H-3′, H-2, H-3, H-4, H-4′, H-3″, H-4″), 3.90 (dd, 1 H, $J$ = 7.0, 3.5 Hz, H-6a‴), 3.61−3.75 (m, 3 H, H-5′, H-5″, H-6$_b$′), 3.74−3.61 (m, 6 H, H-5, H-6$_a$, octyl OC$H_2$, H-6$_b$′, H-6$_b$″, H-6$_b$), 3.54 (app dt, 1 H, $J$ = 10.0, 7.5 Hz, octyl OC$H_2$), 1.60−1.37 (m, 2 H, octyl C$H_2$), 1.37−1.25 (m, 10 H, octyl C$H_2$), 0.88 (t, 3 H, $J$ = 7.0 Hz, octyl C$H_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 108.7 (C-1′), 108.0 (C-1), 107.9 (C-1″), 83.9 (C-4″), 83.8 (C-4), 82.3 (C-4′), 82.1 (C-2′), 81.8 (C-2), 81.2 (C-2″), 77.7 (C-3′), 77.6 (C-3″), 77.3 (C-3), 76.8 (C-5), 71.7 (C-5′), 70.4 (C-5″), 70.2 (C-6′), 69.6 (octyl O*C*$H_2$), 63.8 (C-6), 61.9 (C-6″), 32.0 (octyl *C*$H_2$), 29.5 (octyl *C*$H_2$), 29.3 (octyl *C*$H_2$), 29.2 (octyl *C*$H_2$), 26.1 (octyl *C*$H_2$), 22.9 (octyl *C*$H_2$), 14.5 (octyl *C*$H_3$). ESI-MS *m/z* calcd for [M + Na]$^+$ $C_{26}H_{48}O_{16}Na$: 639.2837. Found: 639.2835.

**Octyl *β*-D-Galactofuranosyl-(1→5)-*β*-D-galactofuranosyl-(1→6)-*β*-D-galactofuranoside (4).** Using the general deprotection procedure, octyl glycoside **41** (60.3 mg, 0.036 mmol) gave a crude product, which was purified by column chromatography (3:1 $CH_2Cl_2$–MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **4** as a clear, colorless oil (20.0 mg, 89%). $R_f$ 0.24 (3:1 $CH_2Cl_2$–MeOH); $[\alpha]_D$ −88.0 (*c* 0.9, MeOH); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$): 5.21 (d, 1 H, $J$ = 1.9 Hz, H-1″), 5.00 (s, 1 H, H-1′), 4.98 (d, 1 H, $J$ = 2.2 Hz, H-1), 4.16 (dd, 1 H, $J$ = 4.0, 1.9 Hz, H-2″), 4.14−4.13 (m, 2 H, H-2′, H-3′), 4.10−4.04 (m, 4 H, H-3″, H-4′, H-3, H-2), 4.02 (dd, 1 H, $J$ = 4.0, 3.0 Hz, H-4″), 3.96−3.94 (m, 3 H, H-4, H-5′, H-5), 3.86−3.71 (m, 6 H, H-6$_a$, H-6$_b$, H-6$_a$′, H-6$_b$′, H-6$_a$″, H-5″), 3.66 (dd, 1 H, $J$ = 12.0, 8.0 Hz, H-6$_b$″), 3.60 (app dt, 1 H, $J$ = 10.0, 7.0 Hz, octyl OC$H_2$), 3.55 (app dt, 1 H, $J$ = 10.0, 7.0 Hz, octyl OC$H_2$), 1.60−1.37 (m, 2 H, octyl C$H_2$), 1.37−1.25 (m, 10 H, octyl C$H_2$), 0.88 (t, 3 H, $J$ = 7.0 Hz, octyl C$H_3$); $^{13}$C NMR (125 MHz, $D_2O$ $\delta_C$): 108.6 (C-1′), 108.0 (C-1), 107.9 (C-1″), 83.5 (C-4′), 83.3 (C-4), 82.7 (C-4″), 82.1 (C-

2″), 82.0 (C-2′), 81.9 (C-2), 77.5 (C-3), 77.4 (C-3″), 77.38 (C-3), 76.8 (C-5′), 71.4 (C-5), 70.2 (C-5″), 70.1 (C-6), 69.4 (octyl O*C*H$_2$), 63.8 (C-6″), 61.9 (C-6′), 32.0 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.9 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). ESI-MS *m*/*z* calcd for (M + Na) $C_{26}H_{48}O_{16}Na$: 639.2837. Found: 639.2835.

**Octyl β-D-Galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranoside (5).** Using the general deprotection procedure, octyl glycoside **45** (11.0 mg, 0.005 mmol) gave a crude product, which was purified by column chromatography (3:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **5** as a clear, colorless oil (3.3 mg, 82%). $R_f$ 0.21 (3:1 $CH_2Cl_2$–MeOH); $[\alpha]_D$ −67.4 (*c* 0.2, MeOH); $^1$H NMR (600 MHz, $D_2O$, $\delta_H$): 5.23 (d, 1 H, *J* = 1.8 Hz, H-1‴), 5.22 (d, 1 H, *J* = 2.0 Hz, H-1′), 5.01 (s, 1 H, H-1″), 4.96 (s, 1 H, *J* = 1.0 Hz, H-1), 4.14−3.94 (m, 12 H, H-2′, H-2″, H-2‴, H-3′, H-3″, H-3‴, H-2, H-3, H-4, H-4′, H-4″, H-4‴), 3.61−3.75 (m, 7 H, H-5′, H-5″, H-6$_a$′, H-6$_a$″, H-6$_a$‴, H-6$_b$‴, H-5‴), 3.74−3.61 (m, 6 H, H-5, H-6$_a$, octyl O*C*H$_2$, H-6$_b$′,H-6$_b$″, H-6$_b$), 3.54 (app dt, 1 H, *J* = 12.0, 7.5 Hz, octyl O*C*H$_2$), 1.60−1.37 (m, 2 H, octyl C*H*$_2$), 1.37−1.25 (m, 10 H, octyl C*H*$_2$), 0.88 (t, 3 H, *J* = 7.0 Hz, octyl C*H*$_3$); $^{13}$C NMR (125 MHz, $D_2O$ $\delta_C$): 108.7 (C-1‴), 108.0, 107.9 (C-1″, C-1′), 107.9 (C-1), 83.9 (C-4″), 85.5 (C-4‴), 82.7 (C-2‴), 82.3 (C-2″), 82.1 (C-4, C-4′), 82.00 (C-2′), 81.9 (C-2), 77.6 (C-3‴), 77.5 (C-3′), 77.4 (C-3″,C-3), 76.8 (C-5″), 76.7 (C-5,C-5‴), 71.4 (C-5′), 70.3 (C-6′), 69.5 (octyl O*C*H$_2$), 63.7 (C-6), 62.0 (C-6″), 61.9 (C-6‴), 32.0 (octyl *C*H$_2$), 30.7 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.9 (octyl *C*H$_2$), 14.3 (octyl *C*H$_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{32}H_{58}O_{21}Na$: 801.3362. Found: 801.3363.

**Octyl β-D-Galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (6).** Using the general deprotection procedure, octyl glycoside **48** (25.0 mg, 0.01 mmol) gave a crude product, which was purified by column chromatography (3:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to furnish **6** as a clear, colorless oil (5.4 mg, 70%). $R_f$ 0.22 (3:1 $CH_2Cl_2$−MeOH,); $[\alpha]_D$ −143.3 (*c* 0.1, MeOH); $^1$H NMR (600 MHz, $D_2O$, $\delta_H$): 5.22 (d, 1 H, *J* = 1.3 Hz, H-1″), 5.04 (d, 1 H, *J* = 1.5 Hz, H-1‴), 5.00 (s, 1 H, H-1′), 4.95 (s, 1 H, H-1), 4.14−3.94 (m, 14 H, H-2″, H-2‴, H-2′, H-3′, H-3″, H-3‴,H-4′, H-3, H-2, H-4″, H-4, H-5′, H-5, H-4‴), 3.90−3.71 (m, 10 H, H-6$_a$, H-6$_b$, H-6$_a$′, H-6$_b$′, H-6$_a$″, H-5″, H-6$_a$‴, H-5‴, H-6$_b$‴, H-6$_b$″), 3.60 (app dt, 1 H, *J* = 10.0, 7.0 Hz, octyl O*C*H$_2$), 3.55 (app dt, 1 H, *J* = 10.0, 7.0 Hz, octyl O*C*H$_2$), 1.60−1.57 (m, 2 H, octyl C*H*$_2$), 1.37−1.25 (m, 10 H, octyl C*H*$_2$), 0.88 (t, 3 H, *J* = 7.0 Hz, octyl C*H*$_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 108.8 (C-1‴), 108.7 (C-1′), 108.0 (C-1), 107.9 (C-1″), 83.9 (C-4‴), 83.83 (C-2‴), 83.81 (C-4′), 83.3 (C-4), 82.8 (C-4″), 82.1 (C-2″), 81.9 (C-2), 77.6 (C-3‴), 77.6 (C-3′), 77.5 (C-3″), 77.4 (C-3), 76.7 (C-5′), 71.72 (C-5‴), 71.69 (C-5), 70.4 (C-5″), 70.0 (C-6″), 69.4, (octyl O*C*H$_2$), 63.6 (C-6), 63.6 (C-6‴), 61.9 (C-6′), 32.0 (octyl *C*H$_2$), 29.0 (octyl *C*H$_2$), 29.6 (octyl *C*H$_2$), 29.3 (octyl *C*H$_2$), 26.1 (octyl *C*H$_2$), 22.9 (octyl *C*H$_2$), 14.5 (octyl *C*H$_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{32}H_{58}O_{21}Na$: 801.3362. Found: 801.3363.

**Octyl α-L-Rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (7).** Using the general deprotection procedure, octyl glycoside **61** (58.0 mg, 0.08 mmol) gave a crude product, which was purified by column chromatography (10:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford disaccharide **7** as a white amorphous solid (22.0 mg, 55%). $R_f$ 0.54 (6:1 $CH_2Cl_2$−MeOH); $[\alpha]_D$ −20.1 (*c* 1.0, MeOH); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$): 4.86 (d, 1 H, *J* = 1.6 Hz, H-1′), 4.82 (d, 1 H, *J* = 3.6 Hz, H-1), 4.05 (dd, 1 H, *J* = 10.4, 3.6 Hz, H-2), 3.98−3.68 (m, 1 H, H-5′), 3.86 (dd, 1 H, *J* = 10.0, 2.2 Hz, H-3′), 3.81−3.68 (m, 6 H, H-3, H-5, H-6$_a$, H-4, octyl O*C*H$_2$, H-2′), 3.57−3.48 (m, 2 H, H-6$_b$, octyl O*C*H$_2$), 3.45 (app t, 1 H, *J* = 10.0 Hz, H-4′), 2.06 (s, 3 H, C(=O)C*H*$_3$), 1.64−1.60 (m, 2 H, octyl C*H*$_2$), 1.38−1.23 (m, 13 H, octyl C*H*$_2$, H-6′), 0.85 (t, 3 H, *J* = 7.0 Hz, octyl C*H*$_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 170.9 (C=O), 102.1 (C-1′), 97.8 (C-1), 80.4 (C-2′), 72.9 (C-4′), 72.7 (C-3′), 71.6 (C-4), 71.1 (C-3), 69.7 (C-5′), 69.2 (C-5), 69.0 (octyl O*C*H$_2$), 61.4 (C-6), 54.2 (C-2), 32.1 (octyl *C*H$_2$), 29.44 (octyl *C*H$_2$), 29.40 (2 × octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 23.0 (octyl *C*H$_2$), 22.8 (C(=O)*C*H$_3$), 17.4 (C-6′), 14.3 (octyl CH$_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{22}H_{41}NO_{10}Na$: 502.2623. Found: 502.2621.

**Octyl β-D-Galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (8).** Using the general deprotection procedure, octyl glycoside **63** (57.3 mg, 0.05 mmol) gave a crude product, which was purified by column chromatography (4:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford trisaccharide **8** as a white amorphous solid (27.2 mg, 89%). $R_f$ 0.36 (3:1 $CH_2Cl_2$−MeOH); $[\alpha]_D$ −13.2 (*c* 0.1, MeOH); $^1$H NMR (500 MHz, $D_2O$, $\delta_H$): 5.29 (br s, 1 H, H-1″), 4.87 (s, 1 H, H-1′), 4.80 (d, 1 H, *J* = 3.5 Hz, H-1), 4.11 (dd, 1 H, *J* = 4.0, 1.8 Hz, H-2″), 4.08−3.99 (m, 4 H, H-3″, H-4″, H-5′, H-2), 3.88−3.48 (m, 13 H, H-3, H-4, H-5, H-6$_a$, H-6$_b$, H-2′, H-3′, H-4′, H-5″, H-6$_a$″, H-6$_b$″, octyl O*C*H$_2$), 2.06 (s, 3 H, C(=O)C*H*$_3$), 1.64−1.60 (m, 2 H, octyl C*H*$_2$), 1.38−1.23 (m, 13 H, octyl C*H*$_2$, H-6′), 0.85 (t, 3 H, *J* = 7.0 Hz, octyl C*H*$_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 174.9 (C=O), 109.1 (C-1″), 101.9 (C-1′), 97.7 (C-1), 83.6 (C-4″), 82.0 (C-2″), 80.5 (C-2′), 78.7 (C-4′), 77.6 (C-3″), 72.9 (C-4), 71.9 (C-3′), 71.5 (C-3), 71.4 (C-5″), 69.1 (C-5′), 69.0 (octyl O*C*H$_2$), 68.3 (C-5), 63.7 (C-6″), 61.4 (C-6), 54.2 (C-2), 32.1 (octyl *C*H$_2$), 29.42 (octyl *C*H$_2$), 29.40 (2 × octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 22.9 (octyl *C*H$_2$), 22.8 (C(=O)*C*H$_3$), 17.4 (C-6′), 14.3 (octyl *C*H$_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{28}H_{51}NO_{15}Na$: 664.3151. Found: 664.3153.

**Octyl β-D-Galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (9).** Using the general deprotection procedure, octyl glycoside **64** (60.0 mg, 0.04 mmol) gave a crude product, which was purified by column chromatography (4:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford tetrasaccharide **9** as a white amorphous solid (21.5 mg, 68%). $R_f$ 0.21 (3:1 $CH_2Cl_2$−MeOH); $[\alpha]_D$ −11.7 (*c* 0.2, MeOH); $^1$H NMR (600 MHz, $D_2O$, $\delta_H$): 5.27 (d, 1 H, *J* = 1.8 Hz, H-1″), 5.22 (d, 1 H, *J* = 1.8 Hz, H-1‴), 4.86 (d, 1 H, *J* = 1.8 Hz, H-1′), 4.80 (d, 1 H, *J* = 3.6 Hz, H-1), 4.15 (dd, 1 H, *J* = 3.6, 1.8 Hz, H-2‴), 4.13−4.02 (m, 7 H, H-3‴, H-4‴, H-2″, H-3″, H-4″, H-5′, H-2), 3.96 (app t, 1 H, *J* = 8.5 Hz, H-3), 3.89−3.63 (m, 15 H, H-4, H-5, H-6$_a$, H-6$_b$, H-2′, H-3′, H-4′, H-5″, H-6$_a$″, H-6$_b$″, H-5‴, H-6$_a$‴, H-6$_b$‴, 2 × octyl O*C*H$_2$), 2.06 (s, 3 H, C(=O)C*H*$_3$), 1.64−1.60 (m, 2 H, octyl C*H*$_2$), 1.38−1.23 (m, 13 H, octyl C*H*$_2$, H-6′), 0.85 (t, 3 H, *J* = 7.0 Hz, octyl C*H*$_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 174.9 (C=O), 109.3 (C-1″), 108.1 (C-1‴), 102.1 (C-1′), 97.7 (C-1), 83.5 (C-4″), 82.5 (C-2‴), 82.4 (C-4‴), 82.1 (C-2″), 80.5 (C-2′), 79.2 (C-4′), 77.4 (C-3″), 77.3 (C-3‴), 76.6 (C-3′), 72.9 (C-4), 71.9 (C-3), 71.5 (C-5″), 71.4 (C-5‴), 69.2 (C-5′), 69.0 (octyl O*C*H$_2$), 68.2 (C-5), 63.7 (C-6″), 62.1 (C-6‴), 61.4 (C-6), 54.2 (C-2), 32.0 (octyl *C*H$_2$), 29.42 (octyl *C*H$_2$), 29.40 (octyl *C*H$_2$), 29.4 (octyl *C*H$_2$), 26.3 (octyl *C*H$_2$), 22.9 (octyl *C*H$_2$), 22.8 (C(=O)*C*H$_3$), 17.4 (C-6′), 14.3 (octyl *C*H$_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{34}H_{61}NO_{20}Na$: 826.3679. Found: 826.3682.

**Octyl β-D-Galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-β-D-galactofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-glucopyranoside (10).** Using the general deprotection procedure, octyl glycoside **65** (46.0 mg, 0.02 mmol) gave a crude product, which was purified by column chromatography (4:3:3:2 EtOAc−AcOH−$CH_3OH$−$H_2O$). The solvent was evaporated and the residue was redissolved in water and lyophilized. The residue obtained was filtered through a Sep-pak C-18 cartridge to afford pentasaccharide **10** as a white amorphous solid (12.0 mg, 62%). $[\alpha]_D$ −2.9 (*c* 0.1, MeOH); $^1$H NMR (600 MHz, $D_2O$, $\delta_H$):

5.26 (d, 1 H, $J = 1.8$ Hz, H-1″), 5.20 (d, 1 H, $J = 1.8$ Hz, H-1‴), 5.04 (d, 1 H, $J = 1.8$ Hz, H-1⁗), 4.86 (s, 1 H, H-1′), 4.79 (br s, 1 H, H-1), 4.14−3.94 (m, 13 H, H-2‴, H-2″, H-3⁗, H-2⁗, H-2, H-3″, H-3‴, H-4‴, H-4⁗, H-5′, H-5″, H-3, H-4), 3.89−3.45 (m, 17 H, H-2′, H-3′, H-4′, H-4″, H-6$_a$, H-6$_b$, H-5, H-6$_a$″, H-6$_b$″, H-5‴, H-6$_a$‴, H-6$_b$‴, H-5⁗, H-6$_a$⁗, H-6$_b$⁗, 2 × octyl OC$H_2$), 2.06 (s, 3 H, C(═O)C$H_3$), 1.64−1.60 (m, 2 H, octyl C$H_2$), 1.38−1.23 (m, 13 H, octyl C$H_2$, H-6′), 0.85 (t, 3 H, $J = 7.0$ Hz, octyl C$H_3$); $^{13}$C NMR (125 MHz, $D_2O$, $\delta_C$): 174.9 (C═O), 109.3 (C-1″), 108.7 (C-1⁗), 108.1 (C-1‴), 102.1 (C-1′), 97.7 (C-1), 83.84 (C-4″), 83.80 (C-4⁗), 82.6 (C-2‴), 82.5 (C-4‴), 82.1 (C-2″), 81.8 (C-2⁗), 80.5 (C-2″), 79.2 (C-4′), 77.6 (C-3″), 77.5 (C-3‴), 77.4 (C-3⁗), 76.5 (C-3′), 72.9 (C-4), 71.9 (C-3), 71.7 (C-5⁗), 71.5 (C-5″), 70.4 (C-5‴), 70.2 (C-6⁗), 69.2 (C-5′), 69.0 (octyl OC$H_2$), 68.3 (C-5), 63.6 (C-6″), 62.1 (C-6‴), 61.4 (C-6), 54.2 (C-2), 32.0 (octyl C$H_2$), 29.4 (octyl C$H_2$), 29.4 (octyl C$H_2$), 29.4 (octyl C$H_2$), 26.3 (octyl C$H_2$), 22.9 (octyl C$H_2$), 22.8 (C(═O)C$H_3$), 17.4 (C-6′), 14.3 (octyl C$H_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{40}H_{71}NO_{25}Na$: 988.4207. Found: 988.4211.

**Octyl α-L-Arabinofuranosyl-(1→5)-β-D-galactofuranoside (11).** Using the general deprotection procedure, octyl glycoside **32** (53.0 mg, 0.05 mmol) gave a crude product, which was purified by column chromatography (6:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **11** as clear, colorless oil (19.3 mg, 90%). $R_f$ 0.31 (4:1 $CH_2Cl_2$−MeOH,); $[\alpha]_D$ −95.0 (*c* 1.0, MeOH); $^1$H NMR (500 MHz, $CD_3OD$, $\delta_H$): 5.19 (d, 1 H, $J =$ 0.5 Hz, H-1′), 4.82 (d, 1 H, $J = 1.8$ Hz, H-1), 4.10 (dd, 1 H, $J =$ 8.7, 5.0 Hz, H-4′), 4.04 (dd, 1 H, $J = 6.5$, 3.0 Hz, H-3), 4.01 (dd, 1 H, $J = 3.0$, 0.5 Hz, H-2′), 3.98 (dd, 1 H, $J = 6.5$, 4.1 Hz, H-4), 3.92−3.89 (m, 3 H, H-6$_a$, H-2, H-5), 3.94 (d, 1 H, $J = 5.0$, 3.0 Hz, H-3′), 3.88−3.77 (m, 4 H, H-6$_b$, H-5$_a$′, octyl OC$H_2$, H-5$_b$′), 3.54 (app dt, 1 H, $J = 9.5$, 7.0 Hz, octyl OC$H_2$), 1.60−1.37 (m, 2 H, octyl C$H_2$), 1.37−1.25 (m, 10 H, octyl C$H_2$), 0.88 (t, 3 H, $J = 7.0$ Hz, octyl C$H_3$); $^{13}$C NMR (125 MHz,$CD_3OD$ $\delta_C$): 109.4 (C-1′), 109.1 (C-1), 86.7 (C-4′), 83.8 (C-4), 83.7 (C-2), 82.5 (C-2′), 78.9 (C-3), 78.8 (C-3′), 77.2 (C-5), 68.9 (octyl OC$H_2$), 63.3 (C-5′), 62.7 (C-6), 33.0 (3 × octyl C$H_2$), 30.72 (octyl C$H_2$), 30.70 (octyl C$H_2$), 27.3 (octyl C$H_2$), 14.4 (octyl C$H_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{19}H_{36}O_{10}Na$: 447.2250. Found: 447.2250.

**Octyl α-L-Arabinofuranosyl-(1→6)-β-D-galactofuranoside (12).** Using the general deprotection procedure, octyl glycoside **33** (80.6 mg, 0.077 mmol) gave a crude product, which was purified by column chromatography (6:1 $CH_2Cl_2$−MeOH). The solvent was evaporated and the residue was redissolved in water before filtration through a Sep-pak C-18 cartridge to afford **12** as a clear, colorless oil (30.0 mg, 92%). $R_f$ 0.31 (4:1 $CH_2Cl_2$−MeOH,); $[\alpha]_D$ −90.0 (*c* 1.0, MeOH); $^1$H NMR (500 MHz, $CD_3OD$, $\delta_H$): 4.91 (s, 1 H, H-1′), 4.82 (d, 1 H, $J = 1.5$ Hz, H-1), 3.99−3.95 (m, 3 H, H-3, H-2′, H-5), 3.94 (d, 1 H, $J = 1.5$ Hz, H-2), 3.88−3.77 (m, 4 H, H-4, H-4′, H-3′, H-5$_a$′), 3.78−3.64 (m, 3 H, H-6$_a$, octyl OC$H_2$, H-6$_b$), 3.54 (dd, 1 H, $J = 11.0$, 3.0 Hz, H-5$_b$′), 3.40 (app dt, 1 H, $J = 9.5$, 7.0 Hz, octyl OC$H_2$), 1.60−1.37 (m, 2 H, octyl C$H_2$), 1.37−1.25 (m, 10 H, octyl C$H_2$), 0.88 (t, 3 H, $J = 7.0$ Hz, octyl C$H_3$); $^{13}$C NMR (125 MHz, $CD_3OD$, $\delta_C$): 110.1 (C-1′), 109.3 (C-1), 86.0 (C-2′), 84.6 (C-2), 83.6 (C-4′), 83.2 (C-4), 78.8 (C-3′), 78.8 (C-3), 71.0 (C-5), 70.6 (C-5′), 68.9 (octyl OC$H_2$), 63.1 (C-6), 33.0 (octyl C$H_2$), 30.7 (octyl C$H_2$), 30.6 (octyl C$H_2$), 30.4 (octyl C$H_2$), 27.3 (octyl C$H_2$), 23.7 (octyl C$H_2$), 14.5 (octyl C$H_3$). ESI-MS *m*/*z* calcd for $[M + Na]^+$ $C_{19}H_{36}O_{10}Na$: 447.2250. Found: 447.2249.

**Acknowledgment.** This work was supported by the Alberta Ingenuity Centre for Carbohydrate Science and the Natural Sciences and Engineering Research Council of Canada. We thank Mr. Ravi Sankar Loka for technical assistance.

**Supporting Information Available:** Details on the synthesis and data for additional new compounds not included above; $^1$H and $^{13}$C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO800457J