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Studies on (β,1→5) and (β,1→6) Linked Octyl Gal$_f$ Disaccharides as Substrates for Mycobacterial Galactosyltransferase Activity

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Abstract—The emergence of multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis* (MTB) and the continuing pandemic of tuberculosis emphasizes the urgent need for the development of new anti-tubercular agents with novel drug targets. The recent structural elucidation of the mycobacterial cell wall highlights a large variety of structurally unique components that may be a basis for new drug development. This publication describes the synthesis, characterization, and screening of several octyl Gal*f*(β,1→5)Gal*f* and octyl Gal*f*(β,1→6)Gal*f* derivatives. A cell-free assay system has been utilized for galactosyltransferase activity using UDP[^{14}C]Gal*f* as the glycosyl donor, and in vitro inhibitory activity has been determined in a colorimetric broth microdilution assay system against MTB H37Ra and three clinical isolates of *Mycobacterium avium* complex (MAC). Certain derivatives showed moderate activities against MTB and MAC. The biological evaluation of these disaccharides suggests that more hydrophobic analogues with a blocked reducing end showed better activity as compared to totally deprotected disaccharides that more closely resemble the natural substrates in cell wall biosynthesis.

Introduction

In spite of the ready availability of highly active agents for the treatment of tuberculosis, this disease remains one of the primary killers worldwide.[1] Furthermore, the resurgence of tuberculosis in developed nations as well as the appearance of multiple drug-resistant forms of the disease throughout the world has raised the concern that this disease may once again resurface as the white plague. These factors have led to an increased awareness of the disease, and public health officials are now calling for increased funding for the development of new drugs and effective vaccines for the treatment of tuberculosis.[2] In particular, there is a critical awareness that new classes of drugs with mechanisms of action that are unique from presently used anti-tubercular agents will be needed to treat drug resistant forms of the disease.[3] In fact, there are few treatment options for patients infected with the highly intractable forms of MDR-TB.[4]

Traditionally, the cell wall of mycobacteria has been one of the major targets for treatment of tuberculosis as is evidenced by the presently accepted mechanism of action of the clinically effective agents isoniazid (INH), ethambutol (EMB), and ethionamide.[5] The value of drugs that target the cell wall is also reinforced by the fact that the waxy nature of the mycobacterial cell wall acts as a protective barrier against both host cellular immune response and typical antibacterial agents.[6] In recent years, the structure of the cell wall has been thoroughly studied in terms of its constituent complex polysaccharides and the specific chemical linkages therein.[5,6b,7] The macromolecular structure of the mycolylarabinogalactan complex has been described.[8] Clues regarding the initiation of mycolylarabinogalactan biosynthesis have arisen from earlier work that showed the arabinogalactan heteropolysaccharide chains are attached through phosphodiester linkages to C-6 of a proportion of muramic acid residues of mycobacterial cell walls.[9] The cell wall core of members of the *Mycobacterium* genus consists of extensively cross linked peptidoglycan to which is attached the linear D-galactan composed of alternating

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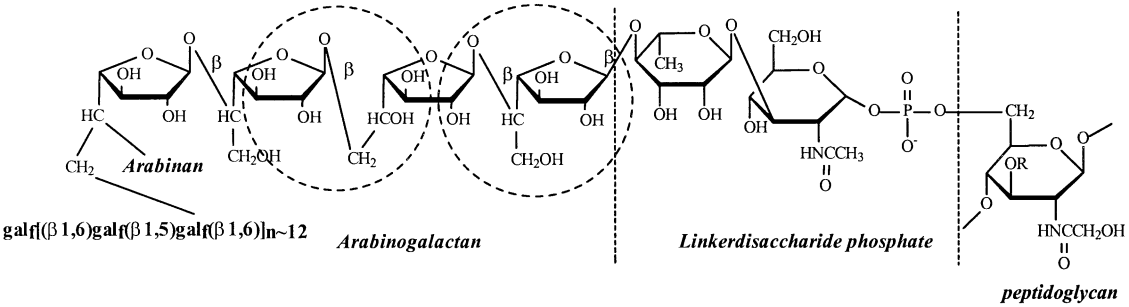
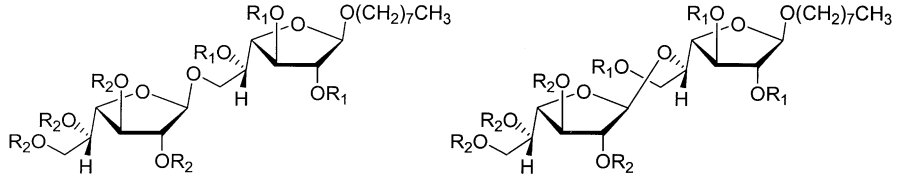


Figure 1. Sugar linkages in cell wall polysaccharide of *Mycobacterium tuberculosis*.



15. R_1=Bz, R_2=Ac
16. R_1=Bn, R_2=Ac
17. R_1=Me, R_2=Ac
18. R_1=Bn, R_2=H
19. R_1=Me, R_2=H
20. R_1=R_2= H

21. R_1=Bz, R_2=Ac
22. R_1=Bn, R_2=Ac
23. R_1=Me, R_2=Ac
24. R_1=Bn, R_2=H
25. R_1=Me, R_2=H
26. R_1=R_2=H

Figure 2. Structures of β(1→6) and β(1→5) linked galactofuranosyl disaccharides.

5- and 6-linked α-D-Gal*f* units.[8b,10a] More recently, chemical analysis of degradation fragments arising from the reducing end of the arabinogalactan obtained from *Mycobacterium tuberculosis, Mycobacterium bovis* BCG and *Mycobacterium leprae* demonstrated the existence of the terminal sequence →5)-D-Gal*f*-(β1→6)-D-Gal*f*-(β1→5)-D-Gal*f*-(β1→4)-L-Rha*p*-(α1→3)-D-GlcNAc (Fig. 1).[10] This unit is crucial to the cell wall infrastructure and anchors the exterior, waxy mycolate units, known targets of the first line agent INH, to the interior peptidoglycan. Since the component sugars L-rhamanose, D-arabinofuranose and D-galactofuranose are not found in mammalian cells, the biosynthesis and utilization of these particular structures offer a new set of drug targets that will be potentially highly active and selective.

Several workers have extensively studied the biosynthesis of the galactofuranose chain in the cell wall.[5,11] The in vitro studies have shown that uridine-5′-diphosphogalactofuranose (UDP-D-Gal*f*) is the substrate for the galactosyltransferases involved in mycobacterial galactan biosynthesis.[11b] The galactosyl residues in the pyranose form are produced through the conversion of UDP-Glc*p* to UDP-Gal*p* by the enzyme UDP-glucose 4-epimerase. UDP-Gal*f* is obtained from UDP-Gal*p* by the unprecedented reaction catalyzed by the enzyme UDP-Gal*p* mutase.[11f–h] The structure of the substrate was confirmed by NMR techniques[11g] and X-ray crystallography.[11i] Again, the absence of D-Gal*f* residues in humans suggests that inhibitors of the enzymes involved either in the formation (mutase) or in the transfer onto the growing galactan chains (galactosyltransferases) may be ideal drug targets.

In a continuation of earlier work,[12] on the synthesis of disaccharides as substrates/inhibitors of mycobacterial glycosyltransferases, we report the synthesis, characterization, and biological evaluation of several galactofuranose disaccharides having β1→5 and β1→6 glycosidic linkages similar to those present in the natural galactan.

Results and Discussion

Synthesis

The β1→6 and β1→5 linked galactofuranosyl disaccharide derivatives (Fig. 2) were synthesized using several glycosylation techniques with different donors (Fig. 3) and acceptors. Crystalline D-(+)-galactofuranose pentaacetate (**1**) was prepared from D-(+)-galactose by a sulfuric acid catalyzed reaction in methanol and subsequent acetylation using acetic anhydride in pyridine.[13] The Lewis acid-mediated glycosylation[14] of **1** using $SnCl_4$ with *n*-octanol yielded octyl 2,3,5,6-tetra-*O*-acetyl-β-D-galctofuranoside (**2**) in 72% yield that on deacetylation with sodium methoxide in methanol gave the octyl β-D-galactofuranoside (**3**) in 95% yield. From compound **3** various acceptors were prepared. Initially,

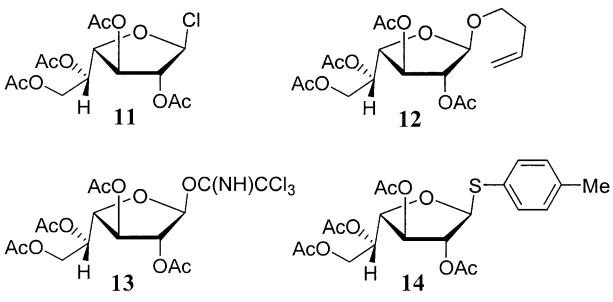
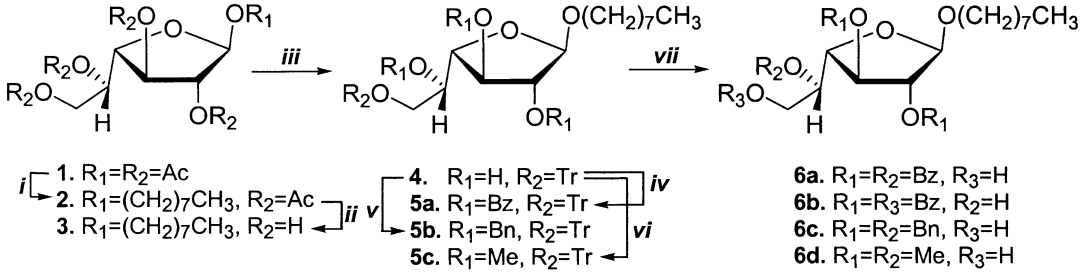


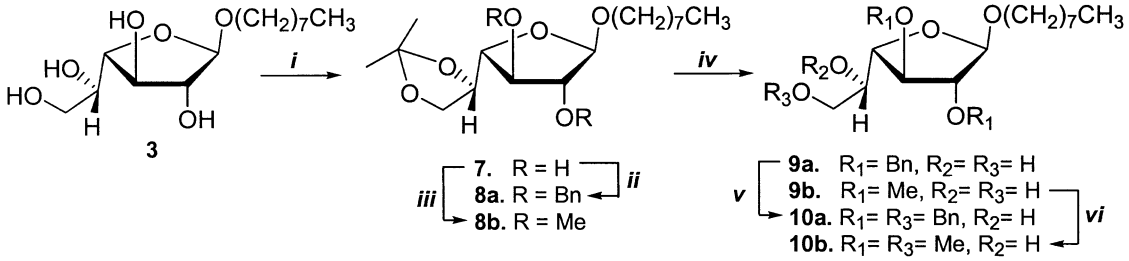
Figure 3. Structure of galactofuranosyl acceptors.

we started with the synthesis of octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (**6a**) and octyl-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (**6b**) as acceptors for the synthesis of β1→6 and β1→5 linked galactofuranose disaccharide derivatives by a route similar to that reported earlier[15] (Scheme 1). Compound **3** was selectively blocked via tritylation with trityl chloride in pyridine at 50 °C to give the 6-tritylated derivative **4** in 72% yield, and **4** was then benzoylated to give **5a** in 87% yield. Treatment with 80% acetic acid gave the mixture of **6a** and **6b** that were separated using column chromatography to give the pure aglycons in 58 and 39% yields, respectively. Each isomer was characterized using ^{1}H and ^{13}C NMR spectroscopy. The byproduct **6b** is formed through migration of the benzoyl group on the 5-position to the 6-hydroxy group during detritylation. The glycosidation reactions with acceptors **6a** and **6b** were carried out with 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl chloride[16] (**11**) as the donor and $HgBr_2$ as the promoter in dichloromethane over 4 Å molecular sieves,[15a] and gave an excellent yield (99%) for the β1→5 linked disaccharide (**21**). An inseparable anomeric mixture [(55:45) of β1→6 (**15**) and β1→5 (**21**) as determined by ^{1}H NMR] was obtained in the coupling reaction of **6a** and **11** to get the β1→6 disaccharide. Hence, a Lewis acid promoted coupling using $SnCl_4$ and 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranose tetraacetate (**1**) as the donor and **6a** and **6b** as acceptors was attempted, but only moderate yields of disaccharides **15** and **21** were obtained.[14] In search for other glycosyl donors, we tried the *n*-pentenyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside[17] (**12**) as the glycosylation donor with acceptors **6a** and **6b** in the presence of NIS and TESOTf/TMSOTf, but no major products were observed even at different reaction temperatures. The use of either NIS/TfOH or NIS/$Sn(OTf)_2$ as acid promoters, however, resulted in an ~79% yield of disaccharide **15**. An attempt to prepare **21** under the same conditions gave an inseparable mixture of disaccharides **15** and **21** in 82% yield in a ratio of 55:45, respectively, and, again, benzoyl migration in acidic medium is probably the cause.

From earlier anti-mycobacterial studies of disaccharides,[12c] it was observed that disaccharides having a fully blocked reducing end gave better activity than the related totally deblocked disaccharides. Hence, we synthesized (Schemes 1 and 2) the protected acceptors **6c**, **6d**, **10a**, and **10b** using benzyl or methyl groups in order to probe steric tolerance in the transferase active site. Compounds **6c** and **6d** were synthesized as described for compound **6a**. Compound **4** was benzylated/methylated using NaH and BnBr/MeI to obtain **5b** and **5c** that on acid hydrolysis (80% acetic acid) gave the aglycon **6c**/**6d** in 78 and 99% yields, respectively. Compounds **10a** and **10b** were prepared starting from **3**. The 5,6-hydroxyl groups were first blocked with an isopropylidene group using 2,2′-dimethoxypropane and (1*S*)-(+)-10-camphor sulphonic acid to yield **7**, and on treatment with benzyl bromide/methyl iodide in presence of NaH afforded compounds **8a** and **8b** in 80 and 81% yields, respectively. Acid hydrolysis, using



Scheme 1. Synthesis of various galactofuranosyl acceptors for β(1→6) disaccharides. Reagents & Conditions: *(i)* $SnCl_4$, $CH_3(CH_2)_7OH$, CH_3CN, 0°C-rt, 30 min, 72%; *(ii)* NaOMe, MeOH, rt, 7 h, 95%; *(iii)* TrCl, Py, 50°C, 48 h, 72%; *(iv)* BzCl, Py, rt, overnight, 87%; *(v)* BnBr, NaH, DMF, 0°C-rt, 3 h, 99%; *(vi)* Mel, NaH, THF, rt, overnight, 90%; *(vii)* CH_3COOH, H_2O, 60°C, 30 min, **6a**: 58%, **6b**: 39%, **6c**: 78%, **6d**: 99%.



Scheme 2. Synthesis of various galactofuranosyl acceptors for β(1→5) disaccharides. Reagents & Conditions: *(i)* 2,2′-dimethoxypropane, (1*S*)-(+)-10-camphorsulphonic acid, acetone, rt, 30 min, 98%; *(ii)* BnBr, NaH, DMF, 0°C-rt, overnight, 80%; *(iii)* Mel, NaH, THF, rt, overnight, 81%; *(iv)* TFA containing 1% H_2O, $CHCl_3$, rt, 30 min, **9a**: 92%, **9b**: 99%; *(v)* Bu_2SnO, Toluene, reflux, overnight, BnBr, CsF, DMF, rt, 8 h, 82%; *(vi)* Bu_2SnO, Toluene, reflux, overnight, Mel, CsF, DMF, rt, overnight, 59%.

Table 1. Inhibitor activities of disaccarides against *Mycobacterium tuberculosis* and *Mycobactrium avium*

Compound no.	MIC[a] (μg/mL)			
	MTB H37Ra	MAC NJ 168	MAC NJ 211	MAC NJ 3404
17	>128	>128	>128	>128
18	>12.8≤128	ND[b]	>12.8≤128	>128
19	>128	>128	>128	>128
20	>128	>128	>128	>128
22	>128	ND	>128	>128
23	>12.8	>12.8	>12.8	>12.8
24	>12.8≤128	ND	>12.8≤128	>128
25	>12.8	>12.8	>12.8	>12.8
26	>128	>128	>128	>128

[a]MIC, minimum inhibitory concentration.
[b]ND, not determined.

trifluoroacetic acid (TFA) containing 1% H_2O in chloroform at room temperature, gave **9a** and **9b** in 92 and 99% respectively. The saccharides **9a** and **9b** were regio-selectively benzylated/methylated via stannylated intermediates[18] formed with dibutyltin oxide in boiling toluene and azeotropic removal of water. Evaporation gave the crude 5,6-*O*-stannylene acetals which were then selectively benzylated/methylated at the 6-positions to give **10a** or **10b** as major products in 82 and 59% yields, as well as minor products **6c** or **6d** in 8 and 10%, respectively, after silica gel flash column chromatography. Each set of isomers was characterized using ^{1}H NMR and ^{13}C NMR spectroscopy. In the carbon spectrum of compound **6c**, C-5 and C-6 resonated at δ 78.23 and 62.30, respectively, whereas in compounds **10a** and **10b** C-5 and C-6 resonated at δ 73.37 and 70.06, respectively. Further, APT and 2D-NMR experiments also supported the assignments of both isomers.

During this work, we investigated other glycosyl donors of galactofuranose for disaccharide synthesis. The trichloroacetimidate derivatives have been widely used as donors for glycosylation reactions in pyranose sugars and recently many furanoses trichloroimidates were reported due to gaining interest in penta- and hexafuranosides.[19] We prepared the 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl trichloroacetaimidate (**13**) in two steps from compound **1** via intermediate 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside. The galactofuranose trichloroacetimidate has recently been prepared and used as glycosyl donor in disaccharide synthesis.[19d,e] The β1→6 and β1→5 linked disaccharides **16** (89%) and **22** (87%) were obtained in high yields using $BF_3{\cdot}Et_2O$ as the promoter in dichloromethane. The only disadvantage to this procedure was a low yield of 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside with HBr/AcOH/H_2O due to ring opening which resulted in some pyranose formation. It was then reacted with trichloroacetonitrile in the presence of K_2CO_3 to yield **13**. We also investigated 1,2-*trans*-thiofuranosyl derivatives of β-D-galactofuranoside as glycosylation donors. Several different sugar donors based on thioethyl or thiophenyl pyranoses and galactofuranoses have been reported and used successfully for different disaccharide preparations.[20] We prepared 1-thiocresyl-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (**14**) as a glycosyl donor, but the use of TMSOTf or TESOTf did not give any disaccharide product in coupling attempts several acceptors. The use of triflic acid (TfOH) or $Sn(OTf)_2$, however, gave excellent yields in short reaction times. We have exclusively used $Sn(OTf)_2$ rather than TfOH due to ease of use and handling for the preparation of the disaccharides **16**, **17**, **22,** and **23**. The structures of these four disaccharides were verified by ^{1}H NMR, ^{13}C NMR and FABMS spectroscopy. H-1 and H-1′ resonated in the range of δ 5.17 and 5.19, respectively, with coupling constants of ~0.7–1.5 Hz whereas the C-1 and C-1′ were observed in the range of δ 105.33 and 105.90, respectively, suggesting the β-glycosidic linkage. Deacytlation of the blocked products with 7N NH_3 in MeOH afforded β1→6 and β1→5 linked disaccharides **18**, **19**, **24,** and **25** having a protected reducing end. Further, catalytic hydrogenation of disaccharides **18** and **24** over Pd/C in MeOH gave the totally deblocked β1→6 and β1→5 linked disaccharides **20** and **26,** respectively. The deacylation of disaccharides **15**, and impure **21**, with 7N NH_3 in MeOH also gave pure, fully deblocked β1→6 and β1→5 linked disaccharides **20** and **26**, respectively, after column chromatography. NMR, FABMS and, whenever necessary, 2D NMR experiments were performed to characterize all compounds.

In-vitro assay

In-vitro antibacterial assays[21] of disaccharides were performed with MTB H_{37}Ra and three clinical isolates of MAC. The results are summarized in Table 1. Moderate activity was seen with **18** and **24** against MTB and one strain of MAC. Both compounds are totally blocked at the reducing end.

Development of a simple mycobacterial galactosyltransferase assay

Based on previous use of specific neoglycolipid acceptors compounds **20** and **26** were synthesized corresponding to the two major structural motifs found within the galactan of arabinogalactan. Assays per-

formed in the presence of membranes and the cell wall enzymatic fraction P60 resulted in excellent [^{14}C]Gal*f* incorporation from UDP-[^{14}C]Gal*p*, following endogenous conversion to UDP-[^{14}C]Gal*f* and transferase activity for both **26** and **20** (see Fig. 4A and B). A concentration of 4 mM for both acceptors resulted in optimum galactosyltransferase activity. TLC/autoradiography (Fig. 5A) demonstrated the enzymatic conversion of both the disaccharide **26** and **20** acceptors to their corresponding trisaccharide products, [^{14}C]Gal to the 5′-OH of 20 and 6′-OH of **26**. Compound **20** gave rise to a second, slower migrating band (Fig. 5A, lane 3) which based on relative migration profiles would be anticipated to be a tetrasaccharide product resulting from further elongation of the above trisaccharide precursor at the 6′-OH consistent with the alternating linkage pattern of arabinogalactan. The complete chemical characterization of the enzymatically synthesized, products along with the identity of the galactosyltransferase gene product are reported in a separate communication.[22] Compounds **18** and **24** were not recognized as substrates for the galactosyltransferase enzyme, presumably due to the bulky benzyl ether protecting groups on the reducing sugar. In contrast, **25** and **19**, which possessed methyl ether protecting groups on the reducing sugar, were recognized as substrates without significant loss of acceptor recognition (Figs 2C, D and 4B). Interestingly, compound **19** produced a single trisaccharide product

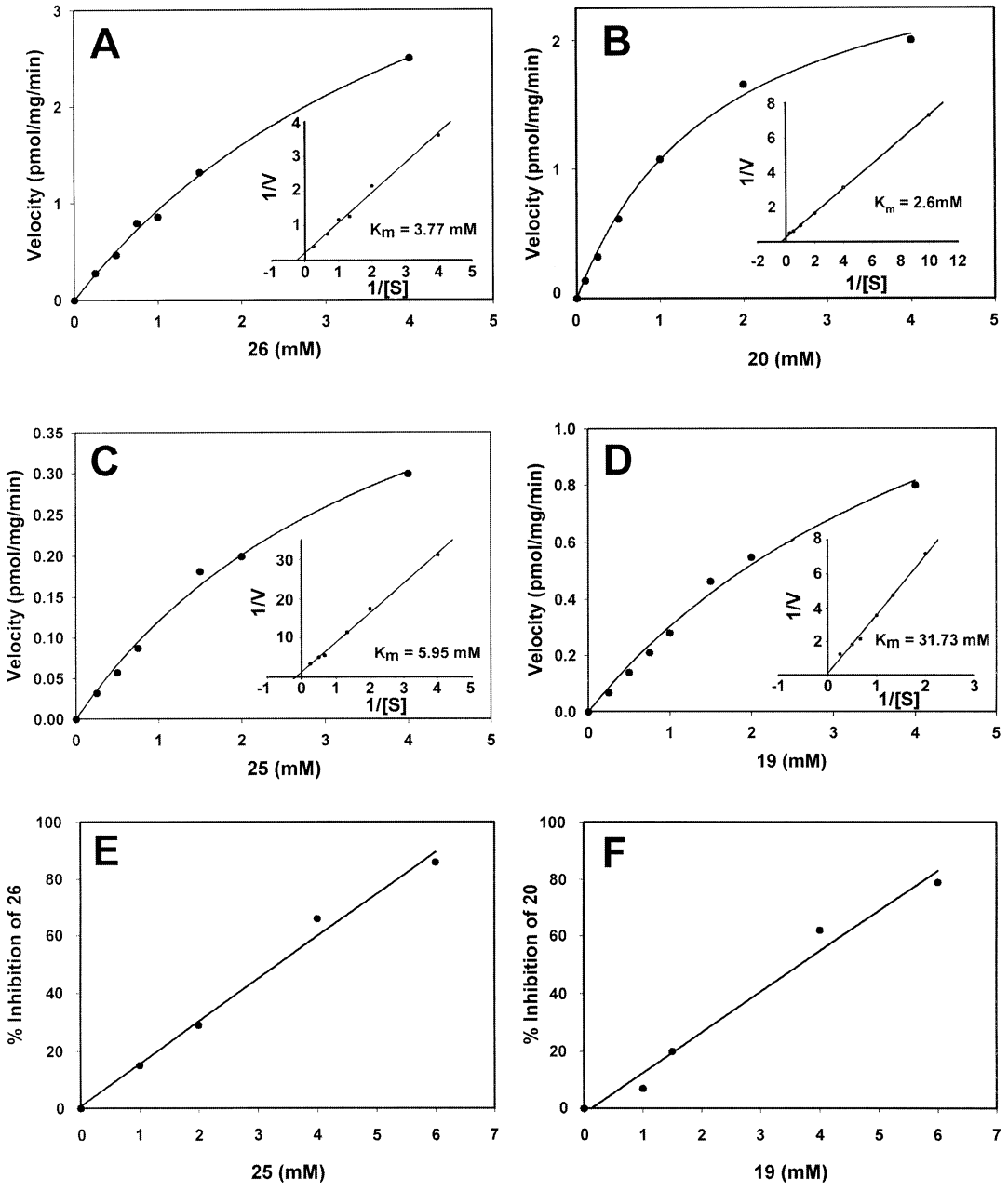


Figure 4. Kinetic analysis of acceptors **26** (panel A), **20** (panel B), **25** (panel C) and **19** (panel D). The insets illustrate the double reciprocal plots for each compound. The inhibitory properties of acceptors **25** (panel E) and **19** (panel F) are shown in a dose–response with compounds **26** and **20** at a fixed concentration of 0.5 mM, respectively.

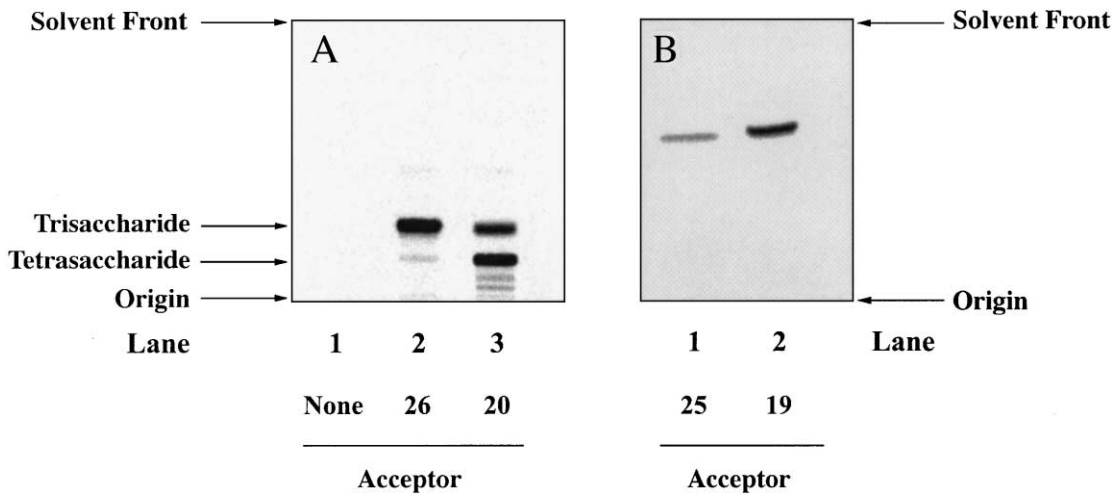


Figure 5. An autoradiogram of reaction products produced through the inclusion of **26** and **20** (panel A), and **25** and **19** (panel B), mycobacterial membranes and UDP-[^{14}C]Gal. TLC/autoradiography was performed using chloroform/methanol/ammonium hydroxide/water (65:25:0.5:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 48 h.

([^{14}C]Gal to the 5′-OH of **19**, whereas the fully deblocked **20** produced a mixture of tri- and tetrasaccharide products as described above (Fig. 2A and B). Calculation of kinetic constants (Fig. 4A–D) revealed that **26** and **20** possessed K_m values 3.77 and 2.60 mM, respectively. In contrast, **25** and **19** possessed K_m values 5.95 and 31.73 mM, respectively. The unexpected high value K_m value for **19**, in addition to its singular product was surprising and may offer some insight into the catalytic mechanism of the bi-functional galactosyltransferase.[22] Further competition based, experiments established that **25** and **19** were effective inhibitors of their native acceptors (**26** and **20**) in the galactosyltransferase assay (Fig. 4E and F) resulting in IC_{50} values of 3.32 and 3.65 mM for **25** and **19**, respectively. The disaccharides **18** and **24** possessed no inhibitory activity in the above assays.

It is intriguing that both **18** and **24** show activity in the in vitro, whole cell assay, but are not active, either as acceptors or inhibitors, in the cell free glycosyltransferase assay. Although, we have not measured the ability of these molecules to bind and lyse cells as nonspecific surfactants, it is possible that both compounds are having activity against the whole bacteria through this mechanism. Clearly, the benzyl groups are too large to allow enzyme binding and inhibition, and they may lead to adverse nonspecific activity as dipolar, uncharged surfactants. The methyl substituted disaccharides, **19** and **25**, on the other hand, appear to compete with the more natural acceptors, **20** and **26**, and show modest IC_{50} values in agreement with our earlier conclusions from octyl β(1→4) Gal*f*-Rha*p* disaccharides;[12c] more hydrophobic disaccharides that are blocked with less sterically demanding blocking groups at the reducing end show acceptor activity as well as binding and inhibition of the mycobacterial galactosyltransferases. These results are in concordance with our efforts to eventually make later generation inhibitors of the mycobacterial glycosyltransferases that are less 'sugar-like' and more 'drug-like.'

Experimental

Chemical synthesis

General procedure. All manipulations were conducted under a dry argon atmosphere. Reaction temperatures were measured externally. Anhydrous solvents from Aldrich were used without further drying. Whenever necessary, compounds and starting reagents were dried by azeotropic removal of water with toluene under reduced pressure. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spray with $(NH_4)_2SO_4$ solution (150 g ammonium sulphate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for work up and chromatography were reagent grade from Fisher Scientific. Flash chromatography was carried out on Fischer silica gel 60 (230–400 mesh). Melting points were determined with a Mel-Temp II capillary melting points apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 and 75 MHz, respectively. Certain ^{1}H NMR spectra were recorded on a Bruker Advance 600 System at 600 MHz. The coupling constants (*J*) are reported in Hz, and chemical shifts are reported in ppm (δ) relative to residual solvent peak or internal standard. Microanalyses were performed on a Perkin–Elmer 2400 CHN analyzer. FABMS were recorded on a Varian/MAT 311A double-focusing mass spectrometer either by adding NBA/LiCl.

Octyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (2). To a solution of β-D-galactofuranose pentaacetate[13] **1** (20.0 g, 51.28 mmol) in dry CH_3CN (100 mL) was added $SnCl_4$ (6.0 mL, 51.28 mmol) at 0 °C and the mixture was stirred for 30 min. To the cold solution, *n*-octanol (8.88 mL, 56.40 mmol) was added dropwise over a period of 30 min. It was again stirred for 1 h at room temperature. Celite 10.0 g was added, the suspension was cooled in an ice-water bath, and a saturated aqueous $NaHCO_3$ solution was added dropwise to precipitate tin

salts. After complete precipitation, the mixture was filtered through Celite and washed with chloroform (2×10 mL). Concentration gave syrup that was redissolved in $CHCl_3$ (400 mL). The solution was washed with water (2×50 mL), brine (2×50 mL), dried over Na_2SO_4 and concentrated in vacuo to give a crude oil. Flash chromatography (cyclohexane/EtOAc, 2:1) gave **2** as a colorless oil (16.97 g, 72%). R_f = 0.51 (cyclohexane/EtOAc, 1:1). FAB-MS (LiCl) *m/e* 467 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$) δ 5.39 (1H, ddd, $J_{4,5}$ = 3.5 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 7.3 Hz, H-5), 5.04 (1H, dd, $J_{1,2}$ = 0.7 Hz, $J_{2,3}$ = 2.0 Hz, H-2), 5.01 (1H, s, H-1), 4.99 (1H, ddd, $J_{1,3}$ = 0.3 Hz, $J_{2,3}$ = 2.0 Hz, $J_{3,4}$ = 5.8 Hz, H-3), 4.34 (1H, dd, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6a), 4.24 (1H, dd, $J_{4,5}$ = 3.5 Hz, $J_{3,4}$ = 5.8 Hz, H-4), 4.22 (1H, dd, $J_{5,6b}$ = 7.3 Hz, $J_{6a,6b}$ = 11.9 Hz, H-6), 3.65 (1H, m, OCH_2), 3.42 (1H, m, OCH_2), 2.14 (3H, s, OAc), 2.11 (3H, s, OAc), 2.09 (3H, s, OAc), 2.06 (3H, s, OAc), 1.56 (2H, m, CH_2), 1.28 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.52, 170.07, 170.04, 169.69 (C=O), 105.48 (C-1), 81.47 (C-2), 79.79 (C-4), 76.64 (C-3), 69.33 (C-5), 67.87 (OCH_2), 62.72 (C-6), 31.84, 29.40, 29.34, 29.27, 26.06, 26.01, 22.68 (6×CH_2), 20.86, 20.81, 20.73 (3×OAc), 14.11 (CH_3).

Octyl β-D-galactofuranoside (3). To a solution of compound **2** (13.5 g, 24.87 mmol) in dry methanol (50 mL) was added NaOMe/MeOH (25% w/v, 9.0 mL) dropwise while cooling in an ice-bath. The reaction mixture was stirred at room temperature for 1 h. Concentration in vacuo gave a syrup. Flash chromatography ($CHCl_3$/MeOH 10:1) gave **3** as a solid (8.57 g, 95%). R_f = 0.31 ($CHCl_3$/MeOH, 7:1), mp 88–90 °C. FAB-MS (NBA) *m/e* 292 $[M+H]^+$. ^1H NMR (300 MHz, DMSO-d_6): δ 5.26 (1H, d, *J* = 5.7 Hz, 2-OH, D_2O exchanged), 5.04 (1H, d, *J* = 5.5 Hz, 3-OH), 4.67 (1H, d, *J* = 2.4 Hz, H-1), 4.51 (1H, 2H, m, 5-OH, 6-OH), 3.81 (1H, dd, $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 7.4 Hz, H-3), 3.74 (1H, dd, $J_{1,2}$ = 2.4 Hz, $J_{2,3}$ = 4.8 Hz, H-2), 3.70 (1H, dd, $J_{4,5}$ = 2.9 Hz, $J_{3,4}$ = 7.4 Hz, H-4), 3.56 (1H, m, OCH_2), 3.49 (1H, m, H-5), 3.31 (3H, m, H_2-6, OCH_2), 1.49 (2H, m, CH_2), 1.25 (10H, m, 5×CH_2), 0.86 (3H, m, CH_3).

Octyl 6-*O*-trityl-β-D-galactofuranoside (4). To a dry pyridine (60 mL) solution of compound **3** (5.2 g, 17.8 mmol) was added trityl chloride (7.44 g, 26.7 mmol), and the reaction mixture was stirred at 50 °C overnight. Co-evaporation with toluene (2×50 mL) removed all traces of pyridine. The resulting oil was redissolved in $CHCl_3$ (150 mL), washed with water (2×20 mL), dried over Na_2SO_4 and concentrated. Column chromatography ($CHCl_3$/MeOH 95:5) gave compound **4** as a pale yellow oil (6.84 g, 72%). R_f = 0.65 ($CHCl_3$/MeOH 95:5). FAB-MS (LiCl) *m/e* 541 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.43 (6H, m, aromatic), 7.30 (9H, m, aromatic), 5.05 (1H, s, H-1), 4.00 (4H, m, H-2, H-3, H-4, H-5), 3.65 (1H, m, OCH_2), 3.37 (3H, m, H_2-6, OCH_2), 2.98 (1H, d, *J* = 11.9 Hz, 3-OH), 2.78 (1H, br s, 2-OH), 2.17 (1H, s, 5-OH) 1.57 (2H, m, CH_2), 1.24 (10H, m, 5×CH_2), 0.86 (3H, m, CH_3).

Octyl 2,3,5-tri-*O*-benzoyl-6-*O*-trityl-β-D-galactofuranoside (5a). Compound **4** (1.00 g, 1.87 mmol) was dissolved in 25 mL of dry pyridine, and benzoyl chloride (1.1 mL, 9.36 mmol) was added. The reaction mixture was stirred overnight at room temperature, poured into an ice–water mixture (25 mL), and extracted with $CHCl_3$ (2×25 mL). The combined $CHCl_3$ layers were washed with brine, dried over Na_2SO_4 and concentrated. Column chromatography (cyclohexane/EtOAc, 10:1) gave pure **5a** (1.36 g, 87%) as a colorless syrup. R_f = 0.80 (cyclohexane/EtOAc 1:1). FAB-MS (LiCl) *m/e* 853 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 8.18–7.16 (30H, m, aromatic), 5.77 (1H, ddd, $J_{4,5}$ = 4.0 Hz, $J_{5,6a}$ = 6.3 Hz, $J_{5,6b}$ = 5.1 Hz, H-5), 5.45 (1H, d, $J_{3,4}$ = 5.3 Hz, H-3), 5.37 (1H, d, $J_{1,2}$ = 1.1 Hz, H-2), 5.20 (1H, s, H-1), 4.72 (1H, dd, $J_{4,5}$ = 4.0 Hz, $J_{3,4}$ = 5.3 Hz, H-4), 3.71 (1H, m, OCH_2), 3.56 (1H, dd, $J_{5,6a}$ = 6.3 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.43 (1H, dd, $J_{5,6b}$ = 5.1 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6b), 3.52–3.47 (1H, m, OCH_2), 1.60–1.55 (2H, m, CH_2), 1.28–1.20 (10H, m, 5×CH_2), 0.90–0.84 (3H, m, CH_3).

Octyl 2,3,5-tri-*O*-benzyl-6-*O*-trityl-β-D-galactofuranoside (5b). Compound **4** (2.36 g, 4.42 mmol) was dissolved in dry DMF (30 mL) and NaH (60% dispersion in mineral oil) was added (382 mg, 15.91 mmol), and the mixture was stirred at room temperature for 15 min. The reaction was cooled to 0°C and benzyl bromide (1.89 mL, 15.91 mmol) was added dropwise, and the solution stirred for 3 h at room temperature. Next, MeOH (10 mL) was added, and the reaction was concentrated to dryness. The resulting oil was redissolved in $CHCl_3$ (200 mL), washed with water (2×50 mL) followed by brine (50 mL), and dried over Na_2SO_4. Concentration of the combined organic layers, and flash chromatography of the product (cyclohexane/EtOAc 20:1) gave compound **5b** as a colorless oil (3.53 g, 99%). R_f = 0.73 (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m/e* 811 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.31 (40H, m, aromatic), 4.97 (1H, s, H-1), 4.54 (6H, m, 3×CH_2-aromatic), 4.11 (1H, dd, *J* = 3.3, 6.8 Hz, H-4), 3.92 (2H, m, H-2, H-3), 3.66 (1H, m, H-5), 3.58 (1H, m, OCH_2), 3.40 (1H, dd, $J_{5,6a}$ = 6.6 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a) 3.31 (2H, m, H-6b, OCH_2), 1.56 (2H, m, CH_2), 1.25 (10H, m, 5×CH_2), 0.87 (3H, m, CH_3).

Octyl 2,3,5-tri-*O*-methyl-6-*O*-trityl-β-D-galactofuranoside (5c). Compound **4** (1.16 g, 2.17 mmol) was dissolved in dry THF (20 mL) and NaH (60% dispersion in mineral oil, 157 mg, 6.52 mmol) was added and the mixture was stirred at room temperature for 15 min. The reaction was cooled to 0 °C and methyl iodide (0.41 mL, 6.52 mmol) was added dropwise to above solution and stirred overnight at room temperature. Methanol (10 mL) was added, and the solution was concentrated to dryness. The resulting oil was redissolved in $CHCl_3$ (150 mL), washed with water (2×25 mL), brine (25 mL) and dried over Na_2SO_4. Concentration of the combined organic layers followed by flash chromatography (cyclohexane/EtOAc 5:1) gave compound **5c** as a colorless oil (1.12 g, 90%). R_f = 0.55 (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m/e* 583 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.46 (6H, m, aromatic), 7.28 (12H, m, aromatic), 4.93 (1H, s, H-1), 4.01 (1H, dd, $J_{4,5}$ = 3.9 Hz, $J_{3,4}$ = 7.0 Hz, H-4), 3.65 (1H,

dd, $J_{1,2}=1.3$ Hz, $J_{2,3}=3.1$ Hz, H-2), 3.59 (2H, m, H-3, OCH_2), 3.51 (3H, s, OCH_3), 3.47 (1H, m, H-5), 3.35 (2H, m, H-6a, OCH_2), 3.36, 3.31 (each 3H, s, $2\times OCH_3$), 3.21 (1H, dd, $J_{5,6b}=5.1$ Hz, $J_{6a,6b}=9.7$ Hz, H-6b), 1.53 (2H, m, CH_2), 1.23 (10H, m, $5\times CH_2$), 0.87 (3H, m, CH_3).

Octyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (6a) and Octyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (6b). Compound **5a** (1.36 g, 1.61 mmol) was dissolved in $CHCl_3$, cooled to 0 °C, and trifluoroacetic acid (10% in $CHCl_3$) was added dropwise. The reaction mixture was stirred for 1 h, co-evaporated with ethanol (2×20 mL), and concentrated to give a syrup. Flash chromatography (cyclohexane/EtOAc 10:1) yielded **6a** (566 mg, 58%) and **6b** (376 mg, 39%) as separate, pure and colorless syrups. $R_f=0.48$ (**6a**) and 0.53 (**6b**) (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m*/*e* 611 $[M+Li]^+$. **Compound 6a**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–7.94 (6H, m, aromatic), 7.64–7.26 (9H, m, aromatic), 5.64 (1H, ddd, $J_{5,6a}=J_{5,6b}=4.8$ Hz, $J_{4,5}=4.2$ Hz, H-5), 5.59 (1H, d, $J_{3,4}=4.8$ Hz, H-3), 5.47 (1H, d, $J_{1,2}=0.9$ Hz, H-2), 5.31 (1H, s, H-1), 4.64 (1H, dd, $J_{4,5}=4.2$ Hz, $J_{3,4}=4.8$ Hz, H-4), 4.08 (2H, d, $J_{5,6a}=J_{5,6b}=4.8$ Hz, H-6a, H-6b), 3.77 (1H, m, OCH_2), 3.56 (1H, m, OCH_2), 1.64 (2H, m, CH_2), 1.27 (10H, m, $5\times CH_2$), 0.88 (3H, m, CH_3). **Compound 6b**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08–8.02, 7.65–7.39 (15H, m, aromatic), 5.63 (1H, d, $J_{3,4}=4.8$ Hz, H-3), 5.51 (1H, d, $J_{1,2}=1.1$ Hz, H-2), 5.26 (1H, s, H-1), 4.60 (1H, dd, $J_{5,6a}=7.9$ Hz, $J_{6a,6b}=12.3$ Hz, H-6a), 4.51–4.46 (2H, m, H-5, H-6b), 4.37 (1H, dd, $J_{4,5}=2.4$ Hz, $J_{3,4}=4.8$ Hz, H-4), 3.72 (1H, m, O-CH_2), 3.50 (1H, m, OCH_2), 1.63 (2H, m, CH_2), 1.21 (10H, m, $5\times CH_2$), 0.85 (3H, m, CH_3).

Octyl 2,3,5-tri-*O*-benzyl-β-D-galactofuranoside (6c). Compound **5b** in glacial acetic acid (25 mL) was heated to 60 °C for 30 min. Water (5 mL) was added dropwise while heating, and the reaction mixture was then cooled to room temperature and stirred for another 30 min followed by co-evaporation with ethanol (2×100 mL). Flash chromatography of the resulting oil (cyclohexane/EtOAc 5:1) gave **6c** as a colorless oil (1.84 g, 74%). $R_f=0.48$ (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m*/*e* 659 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34–7.25 (15H, m, aromatic), 5.05 (1H, s, H-1), 4.69–4.39 (6H, m, $3\times CH_2$-aromatic), 4.19 (1H, m, H-5), 4.08 (1H, dd, $J_{4,5}=3.1$ Hz, $J_{3,4}=5.0$ Hz, H-4), 4.00 (1H, d, $J_{1,2}=0.9$ Hz, H-2), 3.68 (4H, m, H-3, H_2-6, OCH_2), 3.39 (1H, m, OCH_2), 2.27 (1H, dd, $J=4.5$, 8.5 Hz, 6-OH), 1.57 (2H, m, CH_2), 1.27 (10H, br s, $5\times CH_2$), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.28, 137.52, 137.40 (C, aromatic), 128.45, 128.37, 128.04, 128.01, 127.99, 127.92, 127.83, 127.72 (CH, aromatic), 105.95 (C-1, $^1J_{CH}=170.9$ Hz), 88.11 (C-2), 83.21 (C-3), 81.77 (C-4), 78.23 (C-5), 72.71, 72.10, 72.06 ($3\times CH_2$-aromatic), 67.75 (O-CH_2), 62.30 (C-6), 31.83, 29.51, 29.36, 29.27, 26.16, 22.65 ($6\times CH_2$), 14.08 (CH_3).

Octyl 2,3,5-tri-*O*-methyl-β-D-galactofuranoside (6d). Prepared from compound **5c** (1.00 g, 1.74 mmol) using the procedure listed for **6a**. Flash chromatography of the product (cyclohexane/EtOAc 2:1) yielded **6d** as a colorless oil (580 mg, 99%). $R_f=0.23$ (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m*/*e* 341 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.99 (1H, s, H-1), 4.08 (1H, dd, $J_{4,5}=5.5$ Hz, $J_{3,4}=6.6$ Hz, H-4), 3.79 (2H, m, H_2-6), 3.69 (3H, m, H-2, H-3, OCH_2), 3.53, 3.43 (each 3H, s, $2\times OCH_3$), 3.42 (2H, m, H-5, OCH_2), 2.35 (1H, dd, $J=4.6$, 8.6 Hz, 6-OH), 1.57 (2H, m, CH_2), 1.27 (10H, m, $5\times CH_2$), 0.88 (3H, m, CH_3).

Octyl 5,6-isopropyledine-β-D-galactofuranoside (7). To a solution of compound **3** (1.50 g, 5.14 mmol) in dry acetone (30 mL) was added 2,2′-dimethoxypropane (0.95 mL, 7.70 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (120 mg, 0.51 mmol) at room temperature. After 30 min of stirring, the pH was adjusted to pH 7 by adding Et_3N. Concentration in vacuo gave the crude oil which was dissolved in $CHCl_3$ (100 mL), washed with saturated $NaHCO_3$ (20 mL) and water (20 mL), dried over Na_2SO_4, and concentrated to an oil. This oil was further purified by flash chromatography (cyclohexane/EtOAc, 5:1) to give **7** as a colorless oil (1.60 g, 94%). $R_f=0.81$ ($CHCl_3$/MeOH, 8:1). FABMS (NBA) *m*/*e* 333 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.02 (1H, s, H-1), 4.36 (1H, dd, $J=1.3$, 1.5, 7.8 Hz, H-5), 4.03 (6H, m, H-2, H-3, H-4, H_2-6, 3-OH), 3.73 (1H, m, OCH_2), 3.44 (1H, m, OCH_2), 2.06 (1H, d, $J=11.9$ Hz, 2-OH), 1.57 (2H, m, CH_2), 1.42, 1.39 (2×3H, s, $2\times CH_3$), 1.28 (10H, m, $5\times CH_2$), 0.88 (3H, m, CH_3), ^{13}C NMR (75 MHz, $CDCl_3$): δ 110.13 (C), 108.38 (C-1), 85.53 (C-2), 78.6 (C-3), 78.04 (C-4), 75.71 (C-5), 67.80 (O-CH_2), 65.71 (C-6), 31.76, 29.46, 29.25, 29.16, 26.06 ($5\times CH_2$), 25.58, 225.52 ($2\times CH_3$), 22.59 (CH_2), 14.03 (CH_3).

Octyl 5,6- isopropyledine-2,3-di-*O*-benzyl-β-D-galactofuranoside (8a). To a dry DMF solution (20 mL) of compound **7** (1.50 g, 4.52 mmol) was added NaH (60% suspension in mineral oil—325 mg, 13.55 mmol) followed by benzyl bromide (1.61 mL, 13.55 mmol) dropwise with cooling at 0 °C. The reaction mixture was stirred at room temperature overnight. MeOH (5 mL) was added to the reaction, the mixture was evaporated to near dryness, and redissolved in $CHCl_3$ (100 mL). It was washed with water (20 mL) and brine (10 mL), dried over Na_2SO_4, and purified by flash column chromatography (cyclohexane/EtOAc 10:1) to give **8a** as a colorless oil (1.83 g, 79%). $R_f=0.55$ (cyclohexane/EtOAc 2:1). FABMS (LiCl) *m*/*e* 519 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.28 (10H, aromatic), 5.05 (1H, s, H-1), 4.52 (4H, m, $2\times CH_2$), 4.18 (1H, ddd, $J_{4,5}=6.6$ Hz, $J_{5,6a}=J_{5,6b}=6.8$ Hz, H-5), 4.01 (1H, dd, $J_{4,5}=6.6$ Hz, $J_{3,4}=6.8$ Hz, H-4), 4.02 (1H, d, $J_{1,2}=1.3$ Hz, H-2), 3.85 (2H, dd, $J_{5,6a}=J_{5,6b}=6.8$ Hz, H-6a, H-6b), 3.76 (2H, m, H-3, OCH_2), 3.41 (1H, m, OCH_2), 1.57 (2H, m, CH_2), 1.41, 1.35 (each 3H, s, $2\times CH_3$), 1.27 (10H, m, $5\times CH_2$), 0.87 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 137.52, 137.46 (C, aromatic), 128.45, 128.36, 127.95, 127.82, 127.74 (CH, aromatic), 109.60 (C), 105.98 (C-1), 88.01 (C-2), 83.99 (C-3), 81.52 (C-4), 76.49 (C-5), 72.07, 72.04 ($2\times CH_2$-aromatic), 67.72 (O–CH_2), 65.37 (C-6), 31.82, 29.52, 29.35, 29.25, 26.11, 22.65 ($6\times CH_2$), 26.48 (CH_3), 25.41 (CH_3), 14.07 (CH_3).

Octyl 5,6-isopropyledine-2,3-di-*O*-methyl-β-D-galactofuranoside (8b). Compound **8b** was prepared by the

procedure of **8a** using **7** (1.75 g, 5.27 mmol) and methyl iodide (1.00 mL, 15.81 mmol). Workup and flash column chromatography (cyclohexane/EtOAc 5:1) gave compound **8b** as a colorless oil (1.79 g, 95%). $R_f = 0.59$ (cyclohexane/EtOAc 1:1). FAB-MS (LiCl) *m/e* 367 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.00 (1H, s, H-1), 4.24 (1H, ddd, $J_{4,5} = 6.2$ Hz, $J_{5,6b} = 6.6$ Hz, $J_{5,6a} = 6.8$ Hz, H-5), 4.01 (1H, dd, $J_{5,6a} = 6.8$ Hz, $J_{6a,6b} = 8.4$ Hz, H-6a), 3.96 (1H, dd, $J_{3,4} = 6.3$ Hz, $J_{4,5} = 6.2$ Hz, H-4), 3.88 (1H, dd, $J_{5,6b} = 6.6$ Hz, $J_{6a,6b} = 8.4$ Hz, H-6b), 3.71 (2H, m, H-2, OCH_2), 3.46 (2H, m, H-3, OCH_2), 3.40 (6H, s, 2×OCH_3), 1.60–1.54 (2H, m, CH_2), 1.45, 1.38 (each 3H, s, 2×CH_3), 1.27 (10H, m, 5×CH_2), 0.90–0.85 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 109.72 (C), 105.80 (C-1), 89.91 (C-2), 86.09 (C-3), 81.77 (C-4), 76.27 (C-5), 67.80 (O-CH_2), 65.48 (C-6), 57.92, 57.44 (2×OCH_3), 31.87, 29.61, 29.39, 29.27 (4×CH_2), 26.51 (CH_3), 26.16 (CH_2), 25.39 (CH_3), 22.66 (CH_2), 14.03 (CH_3).

Octyl 2,3-di-*O*-benzyl-β-D-galactofuranoside (9a). To a solution of compound **8a** (1.75 g, 3.42 mmol) in $CHCl_3$ (25 mL) was added TFA containing 1% H_2O (5 mL) and the reaction mixture was stirred for 30 min. Next, ethanol (50 mL) was added. The solution was concentrated in vacuo, coevaporated with toluene (2×10 mL) to remove traces of TFA, and flash chromatographed (cyclohexane/EtOAc 2:1) to give **9a** as a colorless oil (1.46 g, 91%). $R_f = 0.25$ (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m/e* 479 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37–7.27 (10H, m, aromatic), 5.03 (1H, d, $J_{1,2} = 1.1$ Hz, H-1), 4.55 (4H, m, 2×OCH_2-aromatic), 4.06 (2H, m, H-3, H-4), 4.01 (1H, s, H-2), 3.67 (4H, m, H-5, H_2-6, OCH_2), 3.38 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.28 (13H, m, 5×CH_2), 0.89 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.55, 137.21 (C, aromatic), 128.49, 128.01, 127.86 (CH, aromatic), 106.42 (C-1), 87.58 (C-2), 83.41 (C-3), 82.24 (C-4), 72.35, 72.05 (2×CH_2-aromatic), 71.10 (C-5), 67.87 (O-CH_2), 64.71 (C-6), 31.82, 29.50, 29.35, 29.27, 26.12, 22.65 (6×CH_2), 14.09 (CH_3).

Octyl 2,3-di-*O*-methyl-β-D-galactofuranoside (9b). Prepared from compound **8c** (1.75 g, 5.27 mmol) by the same procedure as reported for compound **9a**. Flash chromatography (cyclohexane/EtOAc 1:1) gave compound **9b** as a colorless oil (1.46 g, 91%). $R_f = 0.25$ (cyclohexane/EtOAc 1:1). FABMS (LiCl) *m/e* 327 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.01 (1H, m, H-1), 4.00 (1H, dd, $J_{5,6a} = 4.6$ Hz, $J_{6a,6b} = 6.4$ Hz, H-6a), 3.82 (1H, ddd, $J_{4,5} = 3.3$ Hz, $J_{5,6a} = 4.6$ Hz, $J_{5,6a} = 8.1$ Hz, H-5), 3.76–3.73 (3H, m, H-3, H-4, H-6b), 3.71 (1H, dd, $J_{1,2} = 0.8$ Hz, $J_{2,3} = 2.5$ Hz, H-2), 3.68–3.63 (1H, m, OCH_2), 3.46–3.39 (1H, m, OCH_2), 3.43, 3.42 (each 3H, s, 2×OCH_3), 1.57 (2H, m, CH_2), 1.28 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 106.00 (C-1), 89.17 (C-2), 85.56 (C-3), 82.57 (C-4), 71.16 (C-5), 67.87 (O-CH_2), 64.68 (C-6), 58.12, 57.47 (2×OCH_3), 31.81, 29.43, 29.32, 29.24, 26.05, 22.65 (6×CH_2), 14.08 (CH_3).

Octyl 2,3,6-tri-*O*-benzyl-β-D-galactofuranoside (10a). A toluene (150 mL) solution of compound **9a** (1.39 g, 2.75 mmol) and dibutyltin oxide (821 mg, 3.3 mmol) was refluxed for 6 h with azeotropic removal of water. The reaction mixture was cooled and evaporated to dryness. The residue was dissolved in dry DMF (10 mL) and to it was anhydrous CsF (835 mg, 5.5 mmol) and benzyl bromide (0.4 mL, 3.3 mmol). The resulting mixture was stirred overnight under argon, then concentrated in vacuo, redissolved in diethylether (150 mL), and washed with 10% aqueous KF solution and water (10 mL). The combined organic phase was dried, concentrated and subjected to flash chromatography (cyclohexane/EtOAc 10:1) to give compound **10a** as a colorless oil (1.31 g, 85%). $R_f = 0.51$ (cyclohexane/EtOAc 2:1). FAB-MS (LiCl) *m/e* 569 $[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35–7.28 (15H, m, aromatic), 5.03 (1H, s, H-1), 4.52 (6H, m, 3×CH_2–aromatic), 4.10 (1H, dd, $J_{4,5} = 3.2$ Hz, $J_{3,4} = 6.6$ Hz, H-4), 4.05 (1H, dd, $J_{2,3} = 2.6$ Hz, $J_{3,4} = 6.6$ Hz, H-3), 4.00 (1H, dd, $J_{1,2} = 1.1$ Hz, $J_{2,3} = 2.6$ Hz, H-2), 3.92 (1H, m, H-5), 3.66 (1H, m, OCH_2), 3.54 (2H, m, H_2-6), 3.38 (1H, m, O-CH_2), 2.40 (1H, d, $J_{5,OH} = 6.4$ Hz, 5-OH), 3.41 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.27 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.06, 137.81, 137.41 (C, aromatic), 128.43, 128.36, 127.94, 127.89, 127.80, 127.74, 127.66 (CH, aromatic), 106.16 (C-1), 87.73 (C-2), 83.25 (C-3), 81.15 (C-4), 73.37 (C-6), 72.20, 71.91, 71.61 (3×CH_2-aromatic), 70.06 (C-5), 67.69 (O-CH_2), 31.83, 29.51, 29.37, 29.27, 26.14, 22.65 (6×CH_2), 14.09 (CH_3).

Octyl 2,3,6-tri-*O*-methyl-β-D-galactofuranoside (10b). From compound **9b** (570 mg, 1.78 mmol) by the same procedure to prepare **10a**. Flash chromatography (cyclohexane/EtOAc 2:1) gave compound **10b** as colorless oil (351 mg, 59%). $R_f = 0.65$ ($CHCl_3$/MeOH 95:5). FAB-MS (LiCl) *m/e* 341$[M+Li]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.00 (1H, s, H-1), 3.98 (1H, dd, $J_{4,5} = 3.5$ Hz, $J_{3,4} = 6.0$ Hz, H-4), 3.90 (1H, dddd, $J_{4,5} = 3.5$ Hz, $J_{5,6a} = 5.3$ Hz, $J_{5,OH} = 5.9$ Hz, $J_{5,6b} = 6.7$ Hz, H-5), 3.74 (1H, ddd, $J_{1,3} = 0.6$ Hz, $J_{2,3} = 2.4$ Hz, $J_{3,4} = 6.0$ Hz, H-3), 3.71 (1H, dd, $J_{1,2} = 0.9$ Hz, $J_{2,3} = 2.4$ Hz, H-2), 3.66 (1H, m, OCH_2), 3.50 (1H, dd, $J_{5,6a} = 5.3$ Hz, $J_{6a,6b} = 9.8$ Hz, H-6a), 3.46 (1H, dd, $J_{5,6b} = 6.7$ Hz, $J_{6a,6b} = 9.8$ Hz, H-6b), 3.43–3.91 (1H, m, OCH_2), 3.42, 3.41, 3.40 (each 3H, s, 3×OAc), 3.16 (1H, d, $J_{5,OH} = 5.9$ Hz, 5-OH), 1.60–1.56 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.90–0.86 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 105.74 (C-1), 89.33 (C-4), 85.42 (C-2), 81.40 (C-3), 74.03 (C-6), 70.12 (C-5), 67.70 (O-CH_2), 59.15, 58.08, 57.41 (3×OCH_3), 31.83, 29.45, 29.34, 29.25, 26.07, 22.66 (6×CH_2), 14.11 (CH_3).

1-Trichloroacetimidoyl-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (13). D-(+)-Galactofuranose pentaacetate (**1**) (2.00 g, 5.1 mmol) was dissolved in 50 mL of glacial acetic acid, followed by dropwise addition of 33% HBr in acetic acid (10 mL) over a 15 min period with cooling (ice/water bath). After complete addition, the reaction mixture was stirred at room temperature for 1 h. Deionized water (5 mL) was added, the reaction was stirred overnight, and was then poured into an ice–water mixture. Next, this mixture was extracted with chloroform, and the combined chloroform extract was washed with

cold saturated aq $NaHCO_3$ followed by cold water. The organic layer was dried and concentrated to syrup. The desired product was purified by Silica gel G (70–230 mesh) column chromatography using cyclohexane/ethyl acetate (2:1) as the eluent to give 1.53 g (73%) of 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside as a mobile syrup. FABMS (LiCl): 355 $(M+Li)^+$. ^1H NMR (mixture of α and β isomers, 1:4): δ 5.52 (1H, dd, $J=1.8$, 4.8 Hz, H-1_α), 5.38 (4H, m, H-1_β, H-2_α, H-5_α, H-5_β), 5.13 (1H, t, $J=5.1$ Hz, H-3_α), 5.05 (2H, m, H-2_β, H-3_β), 4.43 (1H, t, $J=4.6$ Hz, H-4_β), 4.35 (2H, m, H-$6a_{\alpha,\beta}$), 4.19 (2H, m, H-4_α, H-$6b_\beta$), 4.07 (1H, dd, $J=4.6$, 6.4 Hz, H-$6b_\alpha$), 3.48 (1H, dd, $J=2.0$, 6.8 Hz, 1-OH_α), 3.09 (1H, d, $J=3.5$ Hz, 1-OH_β), 2.16, 2.15, 2.14, 2.13, 2.12, 2.10, 2.09, 2.06 (each 3H, s, 8×OAc).

The 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (1.20 g, 3.45 mmol) was dissolved in dry CH_2Cl_2 (30 mL) followed by the addition of anhydrous K_2CO_3 (700 mg) and trichloroacetonitrile (0.86 mL, 8.63 mmol) at room temperature. The reaction mixture was stirred for 6 h at room temperature, filtered through a Celite pad, and concentrated to a syrup. It was purified by Silica gel G (70–230 mesh) column chromatography with cyclohexane/ethyl acetate (2:1) as the eluent to give the desired pure compound **13** as a syrup (1.70 g, 80%). $R_f=0.48$ (cyclohexane/EtOAc 1:1). FAB-MS (LiCl): 498 $(M+Li)^+$. ^1H NMR: δ 8.64 (1H, s, NH), 6.35 (1H, s, H-1), 5.42 (1H, ddd, $J_{4,5}=4.4$ Hz, $J_{5,6a}=4.6$ Hz, $J_{5,6b}=7.2$ Hz, H-5), 5.35 (1H, dd, $J_{1,2}=1.3$ Hz, $J_{2,3}=2.0$ Hz, H-2), 5.11–5.09 (1H, ddd, $J_{1,3}=0.5$ Hz, $J_{2,3}=2.0$ Hz, $J_{3,4}=4.2$ Hz, H-3), 4.46 (1H, dd, $J_{3,4}=4.2$ Hz, $J_{4,5}=4.4$ Hz, H-4), 4.37 (1H, dd, $J_{5,6a}=4.6$ Hz, $J_{6a,6b}=11.9$ Hz, H-6a), 4.21 (1H, dd, $J_{5,6b}=7.2$ Hz, $J_{6a,6b}=11.9$ Hz, H-6b), 2.16, 2.14, 2.10, 2.05 (each 3H, s, 4×OAc).

1-Deoxy-1-thiocresyl-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (14). D-(+)-Galactofuranose pentaacetate (**1**) (4.75 g, 12.17 mmol) was dissolved in dry CH_2Cl_2 (60 mL) and cooled to 0 °C, and *p*-thiocresol (1.77 gm, 14.6 mmol) was then added. After the mixture was stirred for 20 min under argon, $BF_3{\cdot}Et_2O$ (3.85 mL, 30.43 mmol) was added dropwise. The reaction was neutralized after 30 min with Et_3N (4.4 mL, 30.43 mmol), diluted with CH_2Cl_2, and then washed with water (50 mL) and brine (50 mL). The solution was dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography (cyclohexane/EtOAc 3:1) to give **14** (5.13 g, 93%) as a clear syrup. $R_f=0.48$ (cyclohexane/EtOAc 1:1). FAB-MS (LiCl): 461.2 $(M+Li)^+$. Anal. calcd for $C_{21}H_{26}O_9S$: C, 55.49; H, 5.77. Found C, 55.20; H, 5.76. ^1H NMR: δ 7.40–7.36 (2H, d, aromatic), 7.14–7.11 (2H, d, aromatic), 5.43 (1H, d, $J_{1,2}=2.5$ Hz, H-1), 5.39 (1H, ddd, $J_{4,5}=3.8$ Hz, $J_{5,6a}=4.6$ Hz, $J_{5,6b}=7.0$ Hz, H-5), 5.21 (1H, dd, $J_{1,2}=2.5$ Hz, $J_{2,3}=2.7$ Hz, H-2), 5.07 (1H, ddd, $J_{1,3}=0.6$ Hz, $J_{2,3}=2.7$ Hz, $J_{3,4}=6.1$ Hz, H-3), 4.47 (1H, ddd, $J_{1,4}=0.5$ Hz, $J_{4,5}=3.8$ Hz, $J_{3,4}=6.1$ Hz, H-4), 4.32 (1H, dd, $J_{5,6a}=4.6$ Hz, $J_{6a,6b}=11.9$ Hz, H-6a), 4.18 (1H, dd, $J_{5,6b}=7.0$ Hz, $J_{6a,6b}=11.9$ Hz, H-6b), 2.34 (3H, s, CH_3), 2.13, 2.11, 2.10, 2.05 (4×OAc). ^{13}C NMR: δ 170.32, 169.84, 169.76, 169.45 (4×OAc), 138.13, 132.82, 129.65, 129.04 (4× aromatic), 90.54 (C-1), 81.01 (C-4), 79.42 (C-2), 76.38 (C-3), 68.93 (C-5), 62.37 (C-6), 21.01, 20.66, 20.62, 20.54 (CH_3, 4×OAc).

Octyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (15). Compound **6a** (300 mg, 0.50 mmol) and activated, powdered 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (10 mL) were cooled at 0 °C. The glycosylation donor **12** (250 mg, 0.6 mmol) in 5 mL dry CH_2Cl_2 was added dropwise under an Ar atmosphere. The mixture was stirred for 15 min, and NIS (135 mg, 0.6 mmol) followed by $Sn(OTf)_2$ (25 mg, 0.06 mmol) were added to initiate coupling. The reaction mixture was allowed to stir for 30 min at rt, and the reaction was quenched by addition of Et_3N (1 mL), diluted with CH_2Cl_2 (10 mL) and filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (10 mL), followed by washing with saturated aqueous $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by column chromatography (cyclohexane/EtOAc 2:1) to give a mixture of disaccharides **15** and **21** (55:45) as a colorless oil (373 mg, 79%). $R_f=0.60$ (cyclohexane/EtOAc 1:2). FABMS (LiCl) *m/e* 941 $[M+Li]^+$. ^1H NMR (300 MHz, $CDCl_3$, selected peaks for **15**): δ 5.83 (1H, m, ddd, $J_{4,5}=3.6$ Hz, $J_{5,6a}=5.5$ Hz, $J_{5,6b}=6.8$ Hz, H-5), 5.55 (1H, d, $J_{3,4}=4.9$ Hz, H-3), 5.43 (1H, d, $J_{1,2}=1.0$ Hz, H-2), 5.36 (1H, ddd, $J_{4',5'}=3.6$ Hz, $J_{5',6'a}=6.8$ Hz, $J_{5',6'b}=5.5$ Hz, H-5′), 5.27 (1H, s, H-1), 5.09 (1H, s, H-1′), 4.99 (1H, dd, $J_{1',2'}=1.2$ Hz, $J_{2',3'}=1.5$ Hz, H-2′), 4.94 (1H, dd, $J_{2',3'}=1.5$ Hz, $J_{3',4'}=5.6$ Hz, H-3′), 4.59 (1H, dd, $J_{3,4}=4.9$ Hz, $J_{4,5}=3.6$ Hz, H-4), 4.18 (1H, dd, $J_{5',6'a}=6.8$ Hz, $J_{6'a,6'b}=11.9$ Hz, H-6′a), 4.05 (1H, dd, $J_{5,6a}=5.5$ Hz, $J_{6a,6b}=10.5$ Hz, H-6a), 3.88 (1H, dd, $J_{5,6b}=6.8$ Hz, $J_{6a,6b}=10.5$ Hz, H-6b), 2.10, 2.02, 1.99, 1.95 (4×OCH_3).

Octyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-benzyl-β-D-galactofuranoside (16). The synthesis of **16**, starting from donor **14** (1.44 g, 3.42 mmol) and acceptor **6c** (1.60 g, 2.85 mmol), was carried out as described for compound **15** (reaction time = 15 min). Column chromatography (cyclohexane/EtOAc 5:1) gave the expected product as colorless oil (2.26 g, 89%). $R_f=0.49$ (cyclohexane/EtOAc 1:1). FABMS (LiCl) *m/e* 899 $[M+Li]^+$. Anal. calcd for $C_{49}H_{64}O_{15}$: C, 65.90; H, 7.22. Found C, 65.36; H, 7.11. ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.20 (15H, m, aromatic), 5.37 (1H, ddd, $J_{4',5'}=3.9$ Hz, $J_{5',6'a}=4.2$ Hz, $J_{5',6'b}=7.3$ Hz, H-5′), 5.08 (1H, dd, $J_{1',2'}=0.7$ Hz, $J_{2',3'}=2.0$ Hz, H-2′), 5.06 (1H, s, H-1′), 5.04 (1H, d, $J_{1,2}=1.6$ Hz, H-1), 4.99 (1H, ddd, $J_{1',3'}=0.4$ Hz, $J_{2',3'}=2.0$ Hz, $J_{3',4'}=5.7$ Hz, H-3′), 4.70, 4.58, 4.52 (each 1H, d, $J=11.8$ Hz, 3×CH_2–aromatic) 4.48 (2H, d, $J=11.8$ Hz, 3×CH_2-aromatic), 4.31 (1H, d, $J=11.8$ Hz, 3×CH_2-aromatic), 4.28 (1H, dd, $J_{5',6'a}=4.2$ Hz, $J_{6'a,6'b}=11.9$ Hz, H-6′a), 4.23 (1H, dd, $J_{4',5'}=3.9$ Hz, $J_{3',4'}=5.7$ Hz, H-4′), 4.19 (1H, dd, $J_{5',6'b}=7.3$ Hz, $J_{6'a,6'b}=11.9$ Hz, H-6′b), 4.07 (1H, dd, $J_{4,5}=3.0$ Hz, $J_{3,4}=6.7$ Hz, H-4), 4.00 (1H, dd, $J_{2,3}=3.3$ Hz, $J_{3,4}=6.7$ Hz, H-3), 3.97 (1H, dd, $J_{1,2}=1.6$ Hz, $J_{2,3}=3.3$ Hz, H-2), 3.85 (1H, dd, $J_{5,6a}=4.1$ Hz,

$J_{6a,6b}$ = 9.9 Hz, H-6a), 3.76 (1H, ddd, $J_{4,5}$ = 3.0 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 7.3 Hz, H-5), 3.70–3.62 (1H, m, O-CH_2), 3.66 (1H, dd, $J_{5,6b}$ = 7.3 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6b), 3.38 (1H, m, OCH_2), 2.12, 2.08, 2.05, 2.03 (each 3H, s, 4×OAc), 1.59–1.54 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.90–0.85 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.36, 169.92, 169.88, 169.42 (4×C=O), 138.22, 137.78, 137.59 (4×C–aromatic), 128.32, 128.24, 128.19, 128.17, 127.87, 127.81, 127.72, 127.66, 127.57 (CH–aromatic), 105.97 (C-1), 105.73 (C-1′), 88.30 (C-2), 82.59 (C-3), 81.07 (C-2′), 80.38 (C-4), 80.15 (C-4′), 76.48 (C-3′), 76.18 (C-5), 73.37, 71.94, 71.79 (3×CH_2-aromatic), 69.33 (C-5′), 68.07 (C-6), 67.72 (OCH_2), 62.61 (C-6′), 31.76, 29.41, 29.33, 29.21, 26.10, 22.59 (6×CH_2), 20.77, 20.66, 20.60, 20.56 (4×OAc), 14.04 (CH_3).

Octyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-methyl-β-D-galacto-furanoside (17). The synthesis **17**, starting from donor **14** (396 mg, 0.94 mmol) and acceptor **6d** (260 mg, 0.78 mmol), by the procedure described for compound **15** (reaction time = 30 min). Column chromatography (cyclohexane/EtOAc 3:1) gave disaccharide **17** as colorless oil (369 mg, 90%). R_f = 0.40 (cyclohexane/EtOAc 1:1). FAB-MS (LiCl) *m/e* 671 $[M+Li]^+$. Anal. calcd for $C_{31}H_{52}O_{15}$: C, 56.01; H, 7.88. Found C, 56.05; H, 7.76. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.38 (1H, ddd, $J_{4',5'}$ = 3.9 Hz, $J_{5',6'a}$ = 4.2 Hz, $J_{5',6'a}$ = 7.3 Hz, H-5′), 5.08 (2H, br s, H-1′, H-2′), 5.01 (1H, dd, $J_{1',3'}$ = 0.7 Hz, $J_{2',3'}$ = 2.2 Hz, $J_{3',4'}$ = 5.7 Hz, H-3′), 4.99 (1H, br s, H-1), 4.33 (1H, dd, $J_{5',6'a}$ = 4.2 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.27 (1H, dd, $J_{4',5'}$ = 3.9 Hz, $J_{3',4'}$ = 5.7 Hz, H-4′), 4.21 (1H, dd, $J_{5',6'b}$ = 7.3 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 3.99 (1H, m, H-3), 3.82 (1H, dd, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.71–3.64 (3H, m, H-2, H-4, OCH_2), 3.63 (1H, dd, $J_{5,6b}$ = 7.2 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6b), 3.54 (1H, m, H-5), 3.53 (3H, s, 5-OCH_3), 3.41 (1H, m, OCH_2), 3.42, 3.40 (each 3H, s 2-OCH_3, 3-OCH_3), 2.13, 2.11, 2.09, 2.06 (each 3H, s, 4×OAc), 1.56 (2H, m, CH_2), 1.27 (10H, m, 5×CH_2), 0.90–0.85 (3H, m, CH_3).

Octyl 6-*O*-(β-D-galactofuranosyl)-2,3,5-tri-*O*-benzyl-β-D-galactofuranoside (18). To a solution of disaccharide **16** (1.70 g, 1.91 mmol) in dry methanol (25 mL) was added 7 N NH_3/MeOH (5 mL) dropwise, and the reaction mixture was stirred at room temperature overnight. Concentration in vacuo to a syrup, followed by flash chromatography ($CHCl_3$/MeOH 10:1), gave **18** as a low melting solid (1.11 g, 81%). R_f = 0.51 ($CHCl_3$/MeOH, 7:1). Mp 42–44 °C. FAB-MS (LiCl) *m/e* 731 $[M+Li]^+$. Anal. calcd for $C_{23}H_{42}O_{11}$.0.5H_2O: C, 67.08; H, 7.83. Found C, 66.94; H, 7.85. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.20 (m, aromatic), 5.03 (1H, s, H-1), 4.95 (1H, s, H-1′), 4.48 (6H, m, 3×CH_2–aromatic), 4.11 (1H, dd, $J_{3,4}$ = 7.1, $J_{4,5}$ = 3.4 Hz, H-4), 4.06 (1H, dd, $J_{3',4'}$ = 2.4, $J_{4',5'}$ = 2.2 Hz, H-4′), 3.97 (3H, m, H-2′, H-3, H-3′), 3.94 (1H, d, $J_{1,2}$ = 0.9 Hz, H-2), 3.88 (1H, dd, $J_{5,6a}$ = 3.7, $J_{6a,6b}$ = 10.3 Hz, H-6a), 3.81 (1H, m, H-5′), 3.62 (6H, m, H-5, H-6b, H-6′a, H-6′b, OCH_2, OH), 3.37 (2H, m, OCH_2, OH), 3.24, 2.23 (each 1H, br s, OH), 1.57 (2H, m, CH_2), 1.27 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.88, 137.62, 137.44 (C, aromatic), 128.44, 128.38, 128.35, 128.08, 128.06, 127.99, 127.91, 127.83, 127.80 (CH, aromatic), 108.25 (C-1′, $^1J_{CH}$ = 176.9 Hz), 105.98 (C-1), 88.06 (C-2), 87.46 (C-4′), 82.89 (C-3), 80.79 (C-4), 78.70 (C-2′), 78.46 (C-3′), 75.95 (C-5), 72.79, 72.05, 71.97 (3×CH_2–aromatic), 70.67 (C-5′), 67.85 (OCH_2), 67.00 (C-6), 63.94 (C-6′), 31.82, 29.43, 29.36, 29.27, 26.14, 22.65 (6×CH_2), 14.10 (CH_3).

Octyl 6-*O*-(β-D-galactofuranosyl)-2,3,5-tri-*O*-methyl-β-D-galactofuranoside (19). Prepared from compound **17** (236 mg, 0.36 mmol) by the procedure described for **18**. Flash chromatography ($CHCl_3$/MeOH 9:1) gave **19** as a colorless oil (155 mg, 88%). R_f = 0.45 ($CHCl_3$/MeOH, 5:1). FAB-MS (LiCl) *m/e* 503 $[M+Li]^+$. Anal. calcd for $C_{23}H_{44}O_{11}$•0.75H_2O: C, 54.18; H, 8.70. Found C, 54.14; H, 8.54. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.02 (1H, s, H-1′), 4.97 (1H, s, H-1), 4.42 (1H, br s, OH), 4.14 (1H, t, $J_{3',4'}$ = $J_{4',5'}$ = 4.7 Hz, H-4′), 4.05 (1H, m, H-3′), 4.02 (1H, dd, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 4.3 Hz, H-4), 4.01 (1H, d, $J_{2',3'}$ = 2.4 Hz, H-2′), 3.91 (1H, ddd, $J_{4',5'}$ = 4.7 Hz, $J_{5',6'a}$ = 6.3 Hz, $J_{5',6'b}$ = 4.0 Hz, H-5′), 3.90 (1H, dd, $J_{5,6a}$ = 4.1 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 3.86 (1H, br s, OH), 3.79 (1H, dd, $J_{5',6'a}$ = 6.3 Hz, $J_{6'a,6'b}$ = 11.2 Hz, H-6′a), 3.73 (1H, dd, $J_{5',6'b}$ = 4.0 Hz, $J_{6'a,6'b}$ = 11.2 Hz, H-6′b), 3.69 (1H, dd, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.9 Hz, H-2), 3.67 (1H, m, OCH_2), 3.65 (1H, dd, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 7.0 Hz, H-3), 3.58 (1H, dd, $J_{5,6b}$ = 5.1 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6b), 3.50 (3H, s, OMe), 3.50 (1H, ddd, $J_{4,5}$ = 4.3 Hz, $J_{5,6a}$ = 4.1 Hz, $J_{5,6b}$ = 5.1 Hz, H-5), 3.42 (3H, s, OMe), 3.41 (1H, m, OCH_2), 3.40 (3H, s, OMe), 1.95 (1H, br s, OH), 1.57 (2H, m, CH_2), 1.30 (10, m, 5×OCH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): 108.17 (C-1′), 105.40 (C-1), 89.47 (C-2), 85.46 (C-4′), 85.35 (C-3), 80.71 (C-4), 79.96 (C-3′), 79.19 (C-5), 78.06 (C-2′), 71.05 (C-5′), 67.66 (OCH_2), 66.70 (C-6), 63.99 (C-6′), 58.87, 57.93, 57.41 (3×OCH_3), 31.76, 29.30, 29.18, 26.02, 22.59 (6×CH_2), 14.05 (CH_3).

Octyl 6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (20)

Method A. To a solution of disaccharide **15** (150 mg, 0.16 mmol) in dry methanol (10 mL) was added 7 N NH_3/MeOH (5 mL) dropwise, and the reaction mixture was stirred at room temperature for 6 h. Concentration in vacuo, and flash chromatography ($CHCl_3$/MeOH 5:1) gave **20** as a colorless oil (67 mg, 92%). R_f = 0.35 ($CHCl_3$/MeOH 5:1). FAB-MS (LiCl) *m/e* 461 $[M+Li]^+$. Anal. calcd for $C_{20}H_{38}O_{11}$•1/2 H_2O: C, 51.82; H, 8.26. Found C, 51.20; H, 8.58. ^{1}H NMR (600 MHz, D_2O): δ 4.93 (1H, d, $J_{1',2'}$ = 1.3 Hz, H-1′), 4.85 (1H, d, $J_{1,2}$ = 1.6 Hz, H-1), 4.02 (1H, dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.0 Hz, H-2), 3.97 (2H, m, H-3, H-3′), 3.94 (1H, dd, $J_{1',2'}$ = 1.3 Hz, $J_{2',3'}$ = 5.4 Hz, H-2′), 3.90 (1H, dd, $J_{3',4'}$ = 6.0 Hz, $J_{4',5'}$ = 4.2 Hz, H-4′), 3.88 (1H, ddd, $J_{4,5}$ = 4.2 Hz, $J_{5,6a}$ = 3.6 Hz, $J_{5,6b}$ = 7.8 Hz, H-5), 3.82 (1H, dd, $J_{3,4}$ = 6.0 Hz, $J_{4,5}$ = 4.2 Hz, H-4), 3.74 (2H, m, H-5′, H-6a), 3.62 (1H, m, OCH_2), 3.61 (1H, dd, $J_{5',6'a}$ = 4.8 Hz, $J_{6'a,6'b}$ = 11.8 Hz, H-6′a), 3.56 (1H, dd, $J_{5',6'a}$ = 7.3 Hz, $J_{6'a,6'b}$ = 11.8 Hz, H-6′b), 3.53 (1H, dd, $J_{5,6b}$ = 7.8 Hz, $J_{6a,6b}$ = 10.8 Hz, H-6b), 3.36 (1H, m, OCH_2), 1.50 (2H, m, CH_2), 1.21 (10H, m, 5×CH_2), 0.79 (3H, m, CH_3). ^{13}C NMR (75 MHz, D_2O): 108.25 (C-1′), 107.51 (C-1), 83.31, 83.07 (C-4, C-4′), 81.43, 81.24 (C-2,

C-2′), 77.08 (C-3, C-3′), 71.09 (C-5′), 69.87 (C-6), 69.74 (C-5), 68.55 (OCH_2), 63.10 (C-6′), 31.88, 29.38, 29.28, 25.98, 22.67 (6×CH_2), 13.99 (CH_3).

Method B. To a methanol solution (15 mL) of disaccharide **18** (750 mg, 1.00 mmol) was added Pd/C (10%, 300 mg), and the mixture was stirred at room temperature under hydrogen (130 mL, 24 h). The reaction was filtered through a Celite pad and concentrated to give viscous, colorless oil. Flash chromatography ($CHCl_3$/MeOH 5:1) gave **20** as low melting hygroscopic solid (406 mg, 86%).

Octyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (21). Compound **21** was prepared starting with **12** (319 mg, 0.76 mmol) and acceptor **6b** (380 mg, 0.63 mmol) by the method described for compound **15** (reaction time = 30 min). Chromatography (cyclohexane/EtOAc 2:1) gave the **21** as a colorless oil (695 mg, 92%). R_f = 0.60 (cyclohexane/EtOAc 1:2). FAB-MS (LiCl) *m*/*e* 941 $[M+Li]^+$. Anal. calcd for $C_{49}H_{58}O_{18}$•1/2 H_2O: C, 62.34; H, 6.30. Found C, 62.27; H, 6.28. ^{1}H NMR (300 MHz, $CDCl_3$): 8.07 (6H, m, aromatic), 7.51 (9H, m, aromatic), 5.70 (1H, d, $J_{3,4}$ = 5.1 Hz, H-3), 5.50 (1H, d, $J_{1',2'}$ = 1.0 Hz, H-1′), 5.50 (1H, s, H-2), 5.32 (1H, ddd, $J_{4',5'}$ = 3.8 Hz, $J_{5',6'a}$ = 4.4 Hz, $J_{5',6'b}$ = 7.4 Hz, H-5′), 5.24 (1H, s, H-1), 5.23 (1H, dd, $J_{1',2'}$ = 1.0 Hz, $J_{2',3'}$ = 2.4 Hz, H-2′), 4.99 (1H, dd, $J_{2',3'}$ = 2.4 Hz, $J_{3',4'}$ = 5.9 Hz, H-3′), 4.68 (1H, dd, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.63 (1H, dd, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.58 (1H, ddd, $J_{4,5}$ = 3.4 Hz, $J_{5,6a}$ = 4.9 Hz, $J_{5,6b}$ = 5.9 Hz, H-5), 4.46 (1H, dd, $J_{3,4}$ = 5.1 Hz, $J_{4,5}$ = 3.4 Hz, H-4), 4.42 (1H, dd, $J_{3',4'}$ = 5.9 Hz, $J_{4',5'}$ = 3.8 Hz, H-4′), 4.30 (1H, dd, $J_{5',6'a}$ = 4.4 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.14 (1H, dd, $J_{5',6'b}$ = 7.4 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 3.73 (1H, m, OCH_2), 3.51 (1H, m, OCH_2), 2.04, 2.00, 1.96, 1.78 (each 3H, s, 4×OCH_3), 1.62 (2H, m, CH_2), 1.25 (10H, m, 5×CH_2), 0.86 (3H, m, CH_3).

Octyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-benzyl-β-D-galactofuranoside (22). Disaccharide **22** was synthesized starting with donor **14** (540 mg, 1.28 mmol) and acceptor **10a** (600 mg, 1.07 mmol) by the procedure described for compound **15** (reaction time = 15 min). Column chromatography (cyclohexane/EtOAc 5:1) gave **22** as a colorless oil (933 mg, 98%). R_f = 0.55 (cyclohexane/EtOAc 1:1). FAB-MS (LiCl) *m*/*e* 899 $[M+Li]^+$. Anal. calcd for $C_{49}H_{64}O_{15}$: C, 65.98; H, 7.17. Found C, 65.56; H, 7.43. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30 (15H, m, aromatic), 5.47 (1H, s, H-1′), 5.32 (1H, ddd, $J_{4',5'}$ = 4.2 Hz, $J_{5',6'a}$ = 3.7 Hz, $J_{5',6'b}$ = 7.5 Hz, H-5′), 5.19 (1H, d, $J_{1,2}$ = 1.6 Hz, H-1), 5.03 (1H, d, $J_{2',3'}$ = 1.5 Hz, H-2′), 4.96 (1H, dd, $J_{2',3'}$ = 1.5 Hz, $J_{3',4'}$ = 5.4 Hz, H-3′), 4.59–4.47 (6H, m, 3×CH_2–aromatic), 3.52 (1H, dd, $J_{4',5'}$ = 4.2, $J_{3\alpha,4\alpha}$ = 5.4 Hz, H-4′), 4.31 (1H, dd, $J_{5',6'a}$ = 3.7 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.13 (1H, dd, $J_{5',6'b}$ = 7.5 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.12–4.02 (4H, m, H-3, H-4, H-5), 3.99 (1H, dd, $J_{1,2}$ = 1.6 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 3.72–3.61 (3H, m, H_2-6, OCH_2), 3.42–3.34 (1H, m, OCH_2), 2.07, 2.04, 1.96, 1,94 (each 3H, s, 4×OAc), 1.56 (2H, m, CH_2), 1.27 (10H, br s, 5×CH_2), 0.89–0.85 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.44, 170.05, 170.04 (C=O), 138.11, 137.88, 137.45 (C, aromatic), 128.40, 128.38, 128.31, 128.14, 128.08, 127.93, 127.72, 127.64, 127.53, 127.49 (CH, aromatic), 105.90(C-1′), 105.33(C-1), 88.43 (C-4′), 83.65 (C-2′), 81.05 (C-4), 80.59 (C-3′), 80.09 (C-2), 77.20 (C-3), 74.06 (C-5′), 73.39, 72.15, 72.09 (3×CH_2-aromatic), 71.17 (C-6′), 69.51 (C-5), 67.79 (O-CH_2), 63.00 (C-6), 31.85, 29.55, 29.40, 29.28, 26.19, 22.66 (6×CH_2), 20.81, 20.72, 20.63, 20.53 (CH_3, OAc), 14.11 (CH_3).

Octyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-methyl-β-D-galacto-furanoside (23). Disaccharide **23** was synthesized starting with donor **14** (295 mg, 0.70 mmol) and acceptor **10b** (243 mg, 0.58 mmol) by the procedure described for compound **15** (reaction time = 30 min). Column chromatography (cyclohexane/EtOAc 4:1) gave **23** as a colorless oil (478 mg, 99%). R_f = 0.40 (cyclohexane/EtOAc 1:1). FAB-MS (LiCl) *m*/*e* 671 $[M+Li]^+$. Anal. calcd for $C_{31}H_{52}O_{15}$. $\frac{1}{4}$ H_2O: C, 55.63; H, 7.91. Found C, 55.80; H, 7.69. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.42 (1H, ddd, $J_{4',5'}$ = 3.6 Hz, $J_{5',6'a}$ = 4.1 Hz, $J_{5',6'b}$ = 7.6 Hz, H-5′), 5.42 (1H, s, H-1′), 5.17 (1H, d, $J_{2',3'}$ = 1.5 Hz, H-2′), 4.98 (1H, dd, $J_{2',3'}$ = 1.5 Hz, $J_{3',4'}$ = 5.3 Hz, H-3′), 4.97 (1H, d, $J_{1,2}$ = 1.3 Hz, H-1), 4.39 (1H, dd, $J_{4',5'}$ = 3.6 Hz, $J_{3',4'}$ = 5.3 Hz, H-4′), 4.37 (1H, dd, $J_{5',6'a}$ = 4.1 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.20 (1H, dd, $J_{5',6'b}$ = 7.6 Hz, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.01 (1H, ddd, $J_{4,5}$ = 3.5 Hz, $J_{5,6a}$ = 7.0 Hz, $J_{5,6b}$ = 4.4 Hz, H-5), 3.97 (1H, dd, $J_{4,5}$ = 3.5 Hz, $J_{3,4}$ = 6.9 Hz, H-4), 3.74 (1H, dd, $J_{2,3}$ = 3.1 Hz, $J_{3,4}$ = 6.9 Hz, H-3), 3.68 (1H, dd, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.1 Hz, H-2), 3.66 (1H, m, OCH_2), 3.61 (1H, dd, $J_{5,6a}$ = 7.0 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6a), 3.56 (1H, dd, $J_{5,6b}$ = 4.4 Hz, $J_{6a,6b}$ = 10.4 Hz, H-6b), 3.41 (1H, m, OCH_2), 3.40, 3.38, 3.35 (each 3H, s, 3×OCH_3), 2.13, 2.11, 2.09, 2.05 (each 3H, s, 3xOAc), 1.57 (2H, m, CH_2), 1.28 (10H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.44, 170.02, 169.96, 169.29 (4×C=O), 105.35 (C-1), 104.95 (C-1′), 89.96 (C-2), 84.90 (C-3), 80.97, 80.52, 80.10 (C-4, C-2′, C-4′), 76.62 (C-3′), 73.62 (C-5), 73.26 (C-6), 69.41 (C-5′), 67.61 (OCH_2), 62.84 (C-6′), 59.06, 57.63, 57.39 (3×OCH_3), 31.74, 29.41, 29.26, 29.16, 26.04, 22.26 (6×CH_2), 20.74, 20.70, 20.58, 20.49 (4×OAc), 14.01 (CH_3).

Octyl-5-*O*-(β-D-galactofuranosyl)-2,3,6-tri-*O*-benzyl-β-D-galactofuranoside (24). To a solution of disaccharide **22** (1.00 g, 0.11 mmol) in dry methanol (15 mL) was added 7 N NH_3/MeOH (3 mL) dropwise and the reaction mixture was stirred at room temperature for 3 h. Concentration in vacuo and flash chromatography ($CHCl_3$/MeOH 10:1) gave **24** as a colorless oil (810 mg, 99%). R_f = 0.53 ($CHCl_3$/MeOH, 7:1). FAB-MS (LiCl) *m*/*e* 731 $[M+Li]^+$. Anal. calcd for $C_{41}H_{56}O_{11}$•H_2O: C, 66.29; H, 7.87. Found C, 66.59; H, 7.89. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.21 (15H, m, aromatic), 5.34 (1H, s, H-1′), 4.98 (1H, s, H-1), 4.58–4.35 (6H, m, 3×CH_2–aromatic), 4.10 (1H, br s, H-3), 4.04 (3H, m, H-5, H-3′, H-4′), 4.01 (1H, br s, H-2), 3.97 (1H, d, 1.8 Hz, H-2′), 3.83 (2H, m, H-4, H-5′), 3.67 (1H, dd, $J_{5',6'a}$ = 6.1 Hz, $J_{6'a,6'b}$ = 11.3 Hz, H-6′a), 3.57 (4H, m, H-

6a, H-6b, H-6′b, OCH_2), 3.33 (1H, m, OCH_2), 1.54 (2H, m, CH_2), 1.29 (10H, m, CH_2), 0.87 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.74, 137.47, 137.05 (C, aromatic), 128.52, 128.41, 128.37, 128.20, 128.12, 127.81, 127.72, 127.65 (CH, aromatic), 106.83 (C-1′), 105.60 (C-1), 87.90 (C-2), 87.36 (C-4′), 84.56 (C-3), 80.76 (C-4), 78.97, 78.79 (C-2′, C-3′), 73.66 (C-5), 73.44, 72.14, 71.89 (3×CH_2-aromatic), 70.92 (C-5′), 70.00 (C-6), 67.66 (OCH_2), 64.12 (C-6′), 31.82, 29.42, 29.36, 29.25, 26.10, 22.66 (6×CH_2), 14.10 (CH_3).

Octyl 5-*O*-(β-D-galactofuranosyl)-2,3,6-tri-*O*-methyl-β-D-galactofuranoside (25). This product was prepared from compound **23** (220 mg, 0.33 mmol) by the procedure described for compound **24**. Flash chromatography ($CHCl_3$/MeOH 95:5) gave **25** as a colorless oil (152 mg, 92%). R_f = 0.51 ($CHCl_3$/MeOH, 9:1). FABMS (LiCl) *m/e* 503 $[M+Li]^+$. $C_{23}H_{44}O_{11}$.1.33 H_2O: C, 53.16; H, 8.99. Found C, 53.09; H, 8.73. ^1H NMR (300 MHz, $CDCl_3$): δ 5.32 (1H, s, H-1′), 4.98 (1H, s, H-1), 4.15 (1H, m, H-4′), 4.07 (1H, d, $J_{1',2'}$ = 1.0 Hz, H-2′), 4.05 (1H, m, H-5), 4.03 (1H, m, H-3′), 3.97 (1H, m, H-4), 3.91 (1H, m, H-5′), 3.76 (1H, dd, $J_{5',6'a}$ = 6.2 Hz, $J_{6'a,6'b}$ = 11.4 Hz, H-6′a), 3.75 (1H, dd, $J_{5',6'b}$ = 4.5 Hz, $J_{6'a,6'b}$ = 11.4 Hz, H-6′b), 3.69 (1H, d, $J_{1,2}$ = 1.0 Hz, H-2), 3.61 (4H, m, H-3, H_2-6, OCH_2), 3.40, 3.39, 3.37 (each 3H, s, OCH_3), 3.39 (1H, m, OCH_2), 1.56 (2H, m, CH_2), 1.27 (1H, m, 5×CH_2), 0.88 (3H, m, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 107.17 (C-1′), 104.96 (C-1), 89.16 (C-2), 87.44 (C-4′), 86.49 (C-3), 81.03 (C-4), 78.85 (C-2′), 78.71 (C-3′), 73.58 (C-5), 72.71 (C-6), 71.16 (C-5′), 67.64 (OCH_2), 64.02 (C-6′), 59.19, 57.84, 57.55 (3×OCH_3), 31.77, 29.36, 29.29, 29.18, 26.01, 22.60 (6×CH_2), 14.05 (CH_3).

Octyl 5-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (26)

Method A. To a solution of disaccharide **21** (200 mg, 0.21 mmol) in dry methanol (15 mL) was added 7 N NH_3/MeOH (5 mL) and the reaction mixture was stirred at room temperature for 6 h. Concentration in vacuo and flash chromatography ($CHCl_3$/MeOH 5:1) gave **24** as a colorless oil (96 mg, 99%). R_f = 0.45 ($CHCl_3$/MeOH, 2:1). FAB-MS (LiCl) *m/e* 461 $[M+Li]^+$. Anal. calcd for $C_{20}H_{38}O_{11}.H_2O$: C, 50.84; H, 8.10. Found C, 50.89; H, 8.70. ^1H NMR (600 MHz, $CDCl_3$): δ 5.14 (1H, d, $J_{1',2'}$ = 1.8 Hz, H-1′), 4.81 (1H, d, $J_{1,2}$ = 1.8 Hz, H-1), 4.07 (1H, dd, $J_{1,2}$ = 1.8 Hz, $J_{2,3}$ = 3.6 Hz, H-2), 4.02 (1H, dd, $J_{3,4}$ = 6.6 Hz, $J_{4,5}$ = 4.2 Hz, H-4), 3.97 (1H, dd, $J_{3',4'}$ = 6.6 Hz, $J_{4',5'}$ = 4.2 Hz, H-4′), 3.96 (1H, dd, $J_{2',3'}$ = 4.2 Hz, $J_{3',4'}$ = 6.6 Hz, H-3′), 3.93 (1H, dd, $J_{1',2'}$ = 1.8 Hz, $J_{2',3'}$ = 4.2 Hz, H-2′), 3.86 (2H, m, H-3, H-5), 3.74 (1H, ddd, $J_{4',5'}$ = 4.2 Hz, $J_{5',6'a}$ = 4.2 Hz, $J_{5',6'b}$ = 7.2 Hz, H-5′), 3.71 (1H, dd, $J_{5,6a}$ = 7.3 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 3.64 (1H, dd, $J_{5,6b}$ = 4.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 3.63 (1H, dd, $J_{5',6'a}$ = 4.2 Hz, $J_{6'a,6'b}$ = 11.6 Hz, H-6′a), 3.58 (1H, dd, $J_{5',6'b}$ = 7.2 Hz, $J_{6'a,6'b}$ = 11.6 Hz, H-6′b), 3.56 (1H, m, OCH_2), 3.37 (1H, m, OCH_2), 1.50 (2H, m, CH_2), 1.21 (10H, m, 5×CH_2), 0.78 (3H, m, CH_3). ^{13}C NMR (75 MHz, D_2O): 107.71 (C-1′), 107.42 (C-1), 82.81 (C-4′), 81.68 (C-4), 81.58 (C-2, C-2′), 76.74 (C-3, C-3′), 76.15 (C-5), 70.76 (C-5′), 68.61 (OCH_2), 63.09 (C-6), 62.07 (C-6′), 31.78, 29.34, 29.22, 29.16, 25.88, 22.60 (6×CH_2), 13.91 (CH_3).

Method B. To a methanol solution (15 mL) of disaccharide **24** (725 mg, 1.00 mmol) was added Pd/C (10%, 300 mg), and the mixture was stirred at room temperature under hydrogen (105 mL, 10 h). Filtration through a Celite pad and concentration in vacuo gave a viscous, colorless oil. Flash chromatography ($CHCl_3$/MeOH 5:1) gave **26** as low melting, hygroscopic solid (410 mg, 90%).

Biological studies

Method for in-vitro activity. Assays were performed using a colorimetric microdilution broth assay by the previously reported method.[21] Each compound was assayed in duplicate at either 2 $\log_{10}$ or 4 $\log_{10}$ dilutions consisting of 1.28 and 12.8 or 0.128, 1.28, 12.8 and 128 μg/mL respectively. The minimum inhibitory concentration (MIC) was recorded as the lowest drug concentration that inhibited the growth completely.

Bacterial strains and growth conditions. *M. smegmatis* mc^2155 was a generous gift from W. R. Jacobs, Albert Einstein College of Medicine, Bronx, New York.[23] Liquid cultures of *M. smegmatis* were grown at 37 °C in Luria Bertoni (LB) broth medium (Difco) supplemented with 0.05% Tween 80, biomass harvested, washed with phosphate buffered saline (PBS) and stored at −20 °C until further use.

Galactosyl transferases

Preparation of membrane and cell wall enzyme fractions. *M. smegmatis* cells (10 g wet weight) were washed and re-suspended in 30 mL of buffer A, containing 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol and 10 mM $MgCl_2$ at 4 °C and subjected to probe sonication (Soniprep 150, MSE Sanyo Gallenkamp, Crawley, Sussex, UK; 1 cm probe) for a total time of 10 min in 60 s pulses and 90 s cooling intervals between pulses. The sonicate was centrifuged at 27,000*g* for 60 min at 4 °C. The resulting mycobacterial cell wall pellets were re-suspended in buffer A. Percoll (Pharmacia, Sweden) was added to yield a 60% suspension and centrifuged at 27,000*g* for 1 h at 4 °C. The upper, particulate diffuse cell wall enzymatically active (P60) band was collected and washed three times with buffer A and re-suspended in buffer A at a final protein concentration of 10 mg/mL. Membrane fractions were obtained by centrifugation of the 27,000*g* supernatant at 100,000*g* for 1 h at 4 °C. The supernatant was carefully removed and the membranes gently re-suspended in buffer A at a protein concentration of 20 mg/mL. Protein concentrations were determined using the BCA Protein Assay Reagent kit (Pierce Europe, Oud-Beijerland, The Netherlands).

Galactosyltransferase assay. Compounds **18**–**20** and **24**–**26**, at a range of concentrations from 0.25 to 4.0 mM (which were stored as 100 mM ethanol stocks), were

dried under a stream of argon in a microcentrifuge tube (1.5 mL) and placed in a vacuum desiccator for 15 min to remove any residual solvent. The compounds were then re-suspended with the remaining constituents of the galactosyltransferase assay in buffer A. The reaction mixtures for assessing [^{14}C]Gal incorporation consisted of UDP-[U-^{14}C]Gal (Amersham Pharmacia Biotech, 327 mCi/mmol, 0.25 μCi, 10 μL), ATP (1 mM, 5 μL), NADH (100 mM, 8 μL), membranes (250 μg, 12.5 μL) and the cell wall fraction (250 μg, 25 μL) in a final reaction volume of 80 μL. The reaction mixtures were then incubated at 37 °C for 1 h. A $CHCl_3/CH_3OH$ (1:1, 533 μL) solution was then added to the incubation tubes and the entire contents centrifuged at 18,000*g*. The supernatant was recovered and dried under a stream of argon and re-suspended in C_2H_5OH/H_2O (1:1, 1 mL) and loaded onto a pre-equilibrated [C_2H_5OH/H_2O (1:1)] 1 mL Whatman strong anion exchange (SAX) cartridge which was washed with 3 mL of ethanol. The eluate was dried and the resulting products partitioned between the two phases arising from a mixture of *n*-butanol (3 mL) and H_2O (3 mL). The resulting organic phase was recovered following centrifugation at 3,500*g* and the aqueous phase was again extracted twice with 3 mL of *n*-butanol saturated water, the pooled extracts were back-washed twice with water saturated with *n*-butanol (3 mL). The *n*-butanol-saturated water fraction was dried and re-suspended in 200 μL of *n*-butanol. The total cpm of radiolabeled material extractable into the *n*-butanol phase was measured by scintillation counting using 10% of the labeled material and 10 mL of EcoScintA (National Diagnostics, Atlanta, USA). The incorporation of [^{14}C]Gal was determined by subtracting counts present in control assays (incubation of the reaction components in the absence of the compounds). Another 10% of the labeled material was subjected to thin-layer chromatography (TLC) in $CHCl_3/CH_3OH/NH_4OH/H_2O$ (65:25:0.5:3.6) on aluminium-backed Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). The autoradiograms were obtained by exposing TLCs to X-ray film (Kodak X-Omat) for 4–5 days. Competition based experiments were performed by mixing compounds together at various concentrations (**20**, 0.5 mM with **18** or **19** at 1.0, 1.5, 4.0, and 6.0 mM; **26**, 0.5 mM with **24** or **25** at 1.0, 2.0, 4.0 and 6.0 mM) followed by thin-layer chromatography/autoradiography (Fig. 5A and B) as described earlier to determine the extent of product formation.

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References and Notes

1. (a) World Health Organization. In *Anti-tuberculosis Drug Resistance in the World*. The WHO/IUATLD Global Project on Anti-tuberculosis Drug Resistance Surveillance. World Health Organization (Publication #WHO/TB/97.229), Geneva, Switzerland, 1997. (b) Cohn, D. L.; Bustreo, F.; Raviglione, M. C. *Clin. Infect. Dis.* **1997**, *24*, S121. (c) Douglas, J. G.; McLeod, M.-J. *Clin. Pharmacokinet* **1999**, *37*, 127. (d) Efferen, L. S. *Curr. Opin. Pulm. Med.* **1997**, *3*, 131. (e) Basso, L. A.; Blanchard, J. S. *Adv. Exp. Med. Biol.* **1998**, *456*, 115. (f) Bastian, I.; Colebunders, R. *Drugs* **1999**, *58*, 633. (g) Bradford, W. Z.; Daley, C. L. *Infect. Dis. Clin. North Am.* **1998**, *12*, 157. (h) Rattan, A.; Kalia, A.; Ahmad, N. *Emerging Infect. Dis.* **1998**, *4*, 195.

2. http://www.niaid.nih.gov/factsheets/tb.htm, http://www.niaid.nih.gov/factsheets/tbrsch.htm and http://www.who.int/gtb/publications/factsheet/index.htm.

3. (a) Butler, D. *Nature* **2000**, *406*, 670. (b) Yew, W. W.; Chau, C. H. *Drugs Aging* **1997**, *10*, 45. (c) Duncan, K. *Novartis Foundation Symposium* **1998**, *217*, 228. (d) ; Schraufnagel, D. E. *Int. J. Tuberc. Lung Dis.* **1999**, *3*, 651. (e) Mitscher, L. A.; Baker, W. *Med. Res. Res.* **1998**, *28*, 363. (f) Barry, C. E., III; Slayden, R. A.; Sampson, A. E.; Lee, R. E. *Biochem. Pharmacol.* **2000**, *59*, 221.

4. Sung, S.-W.; Kang, C. H.; Kim, Y. T.; Han, S. K.; Shim, Y.-S.; Kim, J. H. *Eur. J. Cardio-Thoracic Surg.* **1999**, *16*, 187.

5. Mikusova, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890.

6. (a) Young, D. B. In *Tuberculosis: Pathogenesis, Protection and Control*; Bloom, B. R., Ed; American Society of Microbiology, Washington, DC, 1994; pp 559–567. (b) Brennen, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29. (c) Maddry, J. A.; Suling, W. J.; Reynolds, R. C. *Res. Microbiol.* **1996**, *147*, 106.

7. (a) McNeil, M.; Wallner, S. J.; Hunter, S. W.; Brennan, P. J. *Carbohydr. Res.* **1987**, *166*, 299. (b) Lee, R. E.; Brennan, P. J.; Besra, G. S. *Curr. Top. Microbiol. Immunol.* **1996**, *215*, 1.

8. (a) Abou-Zeid, C.; Voiland, A.; Michel, G.; Cocito, C. *Eur. J. Biochem.* **1982**, *128*, 363. (b) Besra, G. S.; Khoo, K.-H.; McNeil, M. R.; Dell, A.; Morris, H. R.; Brennan, P. J. *Biochemistry* **1995**, *34*, 4257.

9. Liu, T.-Y.; Gotschlich, E. C. *J. Biol. Chem.* **1967**, *242*, 471.

10. (a) Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734. (b) McNeil, M.; Daffe, M.; Brennan, P. J. *J. Biol. Chem.* **1990**, *265*, 18200. (c) McNeil, M.; Brennan, P. J. *Res. Microbiol* **1991**, *142*, 451.

11. (a) Weston, A.; Stern, R. J.; Lee, R. E.; Nassau, P. M.; Monsey, D.; Martin, S. L.; Scherman, M. S.; Besra, G. S.; Duncan, K.; McNeil, M. R. *Tubercle & Lung Disease* **1998**, *78*, 123. (b) De Arruda, M. V.; Colli, W.; Zingales, B. *Eur. J. Biochem.* **1989**, *182*, 413. (c) Lee, R. E.; Smith, M. D.; Pickering, L.; Fleet, G. W. J. *Tetrahedron Lett.* **1999**, *40*, 8689. (d) Kovensky, J.; McNeil, M.; Sinay, P. *J. Org. Chem.* **1999**, *64*, 6202. (e) Barlow, J. N.; Girvin, M. E.; Blanchard, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 6968. (f) Mikusova, K.; Mikus, M.; Besra, G. S.; Hancock, I.; Brennan, P. J. *J. Biol. Chem.* **1996**, *271*, 7820. (g) Lee, R.; Monsey, D.; Weston, A.; Duncan, K.; Rithner, C.; McNeil, M. *Anal. Biochem.* **1996**, *242*, 1. (h) Shibaev, V. N. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 277. (i) McMahon, S. A.; Leonard, G. A.; Buchanan, L. V.; Giraud, M.-F.; Naismith, J. H. *Acta Crystallogr.* **1999**, *D55*, 399.

12. (a) Maddry, J. A.; Bansal, N.; Bermudez, L. E.; Comber, R. N.; Orme, I. A.; Suling, W. J.; Wilson, L. N.; Reynolds, R. C. *Biorg. Med. Chem. Lett.* **1998**, *8*, 237. (b) Reynolds, R. C.; Bansal, N.; Rose, J.; Friedrich, J.; Suling, W. J.; Maddry, J. A. *Carbohydr. Res.* **1999**, *317*, 164. (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. (d) Pathak, A. K.; Pathak, V.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **2001**, *42*, 979.
13. Chittenden, G. J. F. *Carbohydr. Res.* **1972**, *25*, 35.
14. Pathak, A. K.; El-Kattan, Y. A.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998**, *39*, 1497.
15. (a) Sugawara, F.; Nakayama, H.; Ogawa, T. *Agric. Biol. Chem.* **1986**, *50*, 1557. (b) Veeneman, G. H.; Notermans, S.; Hoogerhout, P.; van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 344.
16. Korytnyk, W.; Mills, J. A. *J. Chem. Soc* **1959**, 636.
17. Velty, R.; Benvegnu, T.; Gelin, M.; Privat, E.; Plusquellec, D. *Carbohydr. Res.* **1997**, *299*, 7.
18. (a) Ogawa, T.; Matsui, M. *Carbohydr. Res.* **1978**, *62*, C1. (b) Hultin, P. G.; Buffie, R. M. *Carbohydr. Res.* **1999**, *322*, 14.
19. (a) Isobe, M.; Takahashi, H.; Goto, T. *Tetrahedron Lett.* **1990**, *31*, 717. (b) Fugedi, P.; Liptak, A.; Nanasi, P. *Carbohydr. Res.* **1982**, *107*, C5. (c) Du, Y.; Pan, Q.; Kong, F. *Synlett* **1999**, 1648. (d) Gelin, M.; Ferrieres, V.; Plusquellec, D. *Carbohydr. Lett.* **1997**, *2*, 381. (e) Choudhury, A. K.; Roy, N. *Carbohydr. Res.* **1998**, *308*, 207.
20. (a) Choudhury, A. K.; Roy, N. *Synlett* **1997**, 105. (b) Gelin, M.; Ferrières, V.; Plusquellec, D. *Eur. J. Org. Chem* **2000**, 1423.
21. Suling, W. J.; Reynolds, R. C.; Barrow, E. W.; Wilson, L. N.; Piper, J. R.; Barrow, W. W. *J. Antimicrob. Chemother.* **1998**, *42*, 811.
22. Kremer, L.; Dover, L.G.; Morehouse, C.; Hitchin, 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**, in press.
23. Snapper, S. B.; Melton, R. E.; Mustafa, S.; Kieser, T.; Jacobs, W. R., Jr. *Mol. Microbiol.* **1990**, *4*, 1911.