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Disaccharide analogs as probes for glycosyltransferases in *Mycobacterium tuberculosis*

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Abstract—Glycosyltransferases (GTs) play a crucial role in mycobacterial cell wall biosynthesis and are necessary for the survival of mycobacteria. Hence, these enzymes are potential new drug targets for the treatment of tuberculosis (TB), especially multiple drug-resistant TB (MDR-TB). Herein, we report the efficient syntheses of Ara*f*(α 1 → 5)Ara*f*, Gal*f*(β 1 → 5)Gal*f*, and Gal*f*(β 1 → 6)Gal*f* disaccharides possessing a 5-*N*,*N*-dimethylaminonaphthalene-1-sulfonamidoethyl (dansyl) unit that were prepared as fluorescent disaccharide acceptors for arabinosyl- and galactosyl-transferases, respectively. Such analogs may offer advantages relative to radiolabeled acceptors or donors for studying the enzymes and for assay development and compound screening. Additionally, analogs possessing a 5-azidonaphthalene-1-sulfonamidoethyl unit were prepared as photoaffinity probes for their potential utility in studying active site labeling of the GTs (arabinosyl and galactosyl) in *Mycobacterium tuberculosis* (MTB). Beyond their preparation, initial biological testing and kinetic analysis of these disaccharides as acceptors toward glycosyltransferases are also presented.

1. Introduction

Tuberculosis continues to be a major public health issue throughout the world.[1] Disease incidence remains high, and there are particular concerns relative to the appearance and increase of multi-drug-resistant forms of tuberculosis (MDR-TB).[2] The emergence of drug-resistance strains of mycobacteria is oftentimes a result of patient non-compliance to treatment protocols, and can be attributed in part to the lengthy treatment times and side effects of current therapies. With advanced HIV disease, immunocompromised patients have a higher risk of developing active tuberculosis—either from new exposure to TB or reactivation of quiescent mycobacteria. Without the aid of an active immune system, treatment can be more difficult and the disease may be more resistant to therapy.[3] TB treatment can be exacerbated in AIDS patients undergoing chronic, combined therapies for HIV and attendant opportunistic diseases due to the large number of different agents and an increased likelihood of drug interactions.[4] MTB is an obligate intracellular pathogen that persists within macrophages in the human host, and these cells are involved in dissemination of infection.[5] Intracellular bacilli are more resistant to treatment due to limited access of drugs to bacteria within macrophages, necessitating chronic treatment with high therapeutic doses of multiple antibiotics lasting six months or longer for effective control and treatment of the disease.[6] For these reasons, recent research in tuberculosis has focused on the identification of new drug targets in TB, with a particular emphasis on druggable targets and the development of new, safer agents for the treatment of this disease.

Among the front line drugs for treatment of TB, two drugs isoniazid (INH) and ethambutol (EMB) target the assembly of the mycobacterial cell wall that is essential for the survival of pathogen.[7] The structure of the cell wall has now been thoroughly elucidated in terms of its essential complex polysaccharides, the specific chemical linkages therein, and the macromolecular structure of the mycolylarabinogalactan complex.[8] The cell wall core of members of the *Mycobacterium* genus consists of an extensively cross-linked peptidoglycan to

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which is attached the linear D-galactan composed of alternating 5- and 6-linked β-D-Gal*f* units. Chemical analysis of the degradation fragments arising from the reducing end of the arabinogalactan (AG) obtained from MTB, *Mycobacterium bovis* BCG, and *Mycobacterium leprae* demonstrated the existence of the terminal sequence (β1→5)-D-Gal*f*-(β1 → 6)-D-Gal*f*-(β1 → 5)-D-Gal*f*-(β1 → 4)-L-Rha*p*-(α1 → 3)-D-Glc*p*NAc. 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. The mycobacterial cell wall oligosaccharide is composed of three major sugars, mannopyranose (Man*p*), galactofuranose (Gal*f*), and arabinofuranose (Ara*f*) in a variety of defined glycosidic linkages. The two major oligosaccharide portions are AG and lipoarabinomannan (LAM). AG is composed of alternate (β1 → 5) and (β1 → 6) linked Gal*f* units, linear (α1 → 5) linked Ara*f*, and branched arabinan hexasaccharide at the terminus that consists of (α1 → 5), (α1 → 3), (β1 → 2) linked Ara*f* units (Fig. 1). LAM is Man*p* capped arabinan. The AG complex is critical for the survival of *Mycobacterium tuberculosis*, and is a critical part of the cell wall barrier that protects the bacillus within the macrophage and functions as an effective barrier to antibiotics that are commonly used in the treatment of typical Gram-positive bacteria.

The assembly of the arabinan portions of cell wall polysaccharides in mycobacteria involves a family of arabinosyltransferases (AraTs) that promote the polymerization of decaprenolphosphoarabinose (DPA) as the donor (represented in Fig. 2). The galactan portion is prepared through the actions of a bifunctional galactosyltransferase (GalT) enzyme preparing both the (β1 → 5) and (β1 → 6) linkages along with another recently characterized enzyme (Rv3782), and both enzymes utilize the donor uridinediphosphate-galactofuranose (UDP-Gal*f*) (represented in Fig. 3).[8,9] UDP-Gal*f* is produced by the conversion of UDP-galactopyranose (UDP-Gal*p*) by the enzyme UDP-Gal*p* mutase. Mycobacterial viability requires an intact arabinan and galactan, and thus compounds that inhibit these glycosyltransferases are both useful biochemical tools as well as potential lead compounds for new anti-tuberculosis agents.

We have pursued disaccharide analogs designed as probes for mycobacterial cell wall biochemistry and, specifically, as potential glycosyltransferase substrates and inhibitors.[10] As an example, photoaffinity probes can play an important role in specific labeling and identification of target biomolecules, and they have been useful in the identification of receptors and binding sites for various ligands and in locating functional sites of macromolecules.[11] Cory et al. utilized the sulfonamide derivatives for N-terminal amino acid labeling of proteins.[12] This technique uses photoactivatable, heterobifunctional reagents, which are gener-

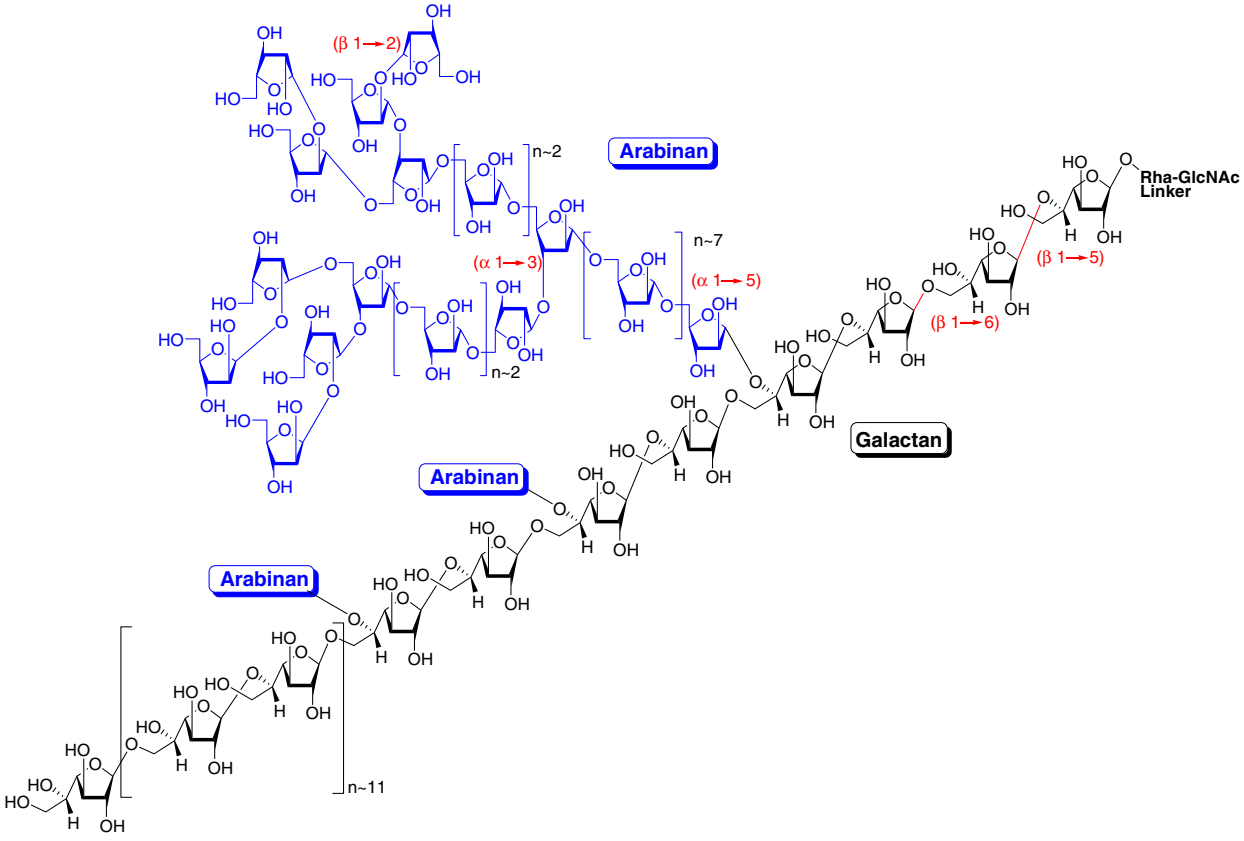


Figure 1. Structure representation of *M. tuberculosis* arabinogalactan (AG) structure.

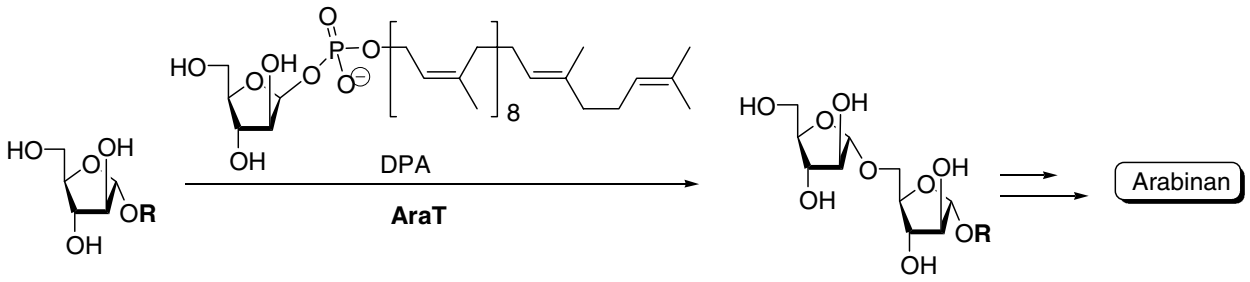


Figure 2. Prototypical arabinosyltransferase-catalyzed reaction.

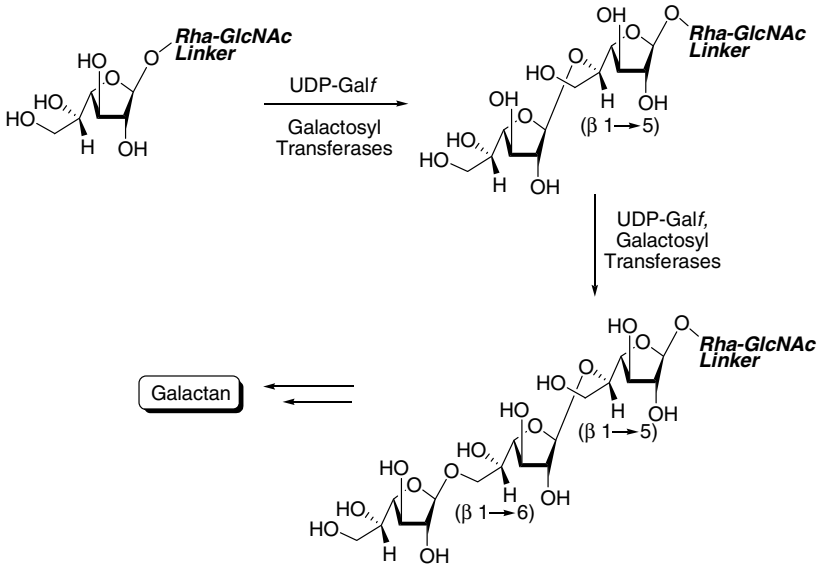


Figure 3. Prototypical galactosyltransferase-catalyzed reaction.

ally azide derivatives that, upon exposure to ultraviolet light, photo decompose into a reactive nitrene intermediate that subsequently forms a cross-link between the ligand and its binding protein under physiological conditions. These adducts can then be analyzed using gel electrophoresis. This technique is potentially useful as a marker because of its high sensitivity and ease of handling.[13]

In the present communication, we would like to provide a detailed account of the synthesis of fluorescent disaccharide probes (**1**–**3**) bearing a highly fluorescent 5-*N*,*N*-dimethylaminonaphthalene-1-sulfonamido[14] (dansyl) function (Chart 1). These substrates were designed for the development of non-radiolabeled second generation competition based acceptor assays that can potentially eliminate the use of radiolabeled donors (DPA and UDP-Gal*p*). Furthermore, this paper describes the preparation of related disaccharides (**4**–**6**) bearing 1-azido-5-naphthalene sulfonamido[13] functionality as potential pro-fluorescent photoaffinity probes to study the nature of AraTs and GalT (Chart 1). Synthetic details and preliminary biological data relating to acceptor activity of these disaccharide probes are discussed.

2. Results and discussion

2.1. Strategy

We have previously described syntheses of Ara*f* and Gal*f* disaccharides based on linkages present in the cell wall of MTB as acceptors/inhibitors of specific mycobacterial glycosyltransferases (GTs).[10] In earlier reports, we briefly communicated the synthesis of fluorescent Ara*f* disaccharides[14] and related photoaffinity probes,[15] and a Man*p* photoaffinity probe[16] as well as acceptor activity for their respective GTs. The present account, while using a similar synthetic strategy, reports detailed methods with modifications for improving the preparation of final targets in both the photoaffinity and fluorescent disaccharide series.

Several different strategies were adopted for Ara*f* disaccharide preparation to introduce an ethylamino functionality for attachment of naphthylsulfonamido group at the anomeric center (Fig. 4). Starting from the readily synthesized Ara*f* thiocresylglycoside **7**, a nitroethyl group was introduced at the anomeric center by reaction with commercially available 1-nitroethanol that afforded nitroethyl glycoside **8** in excellent

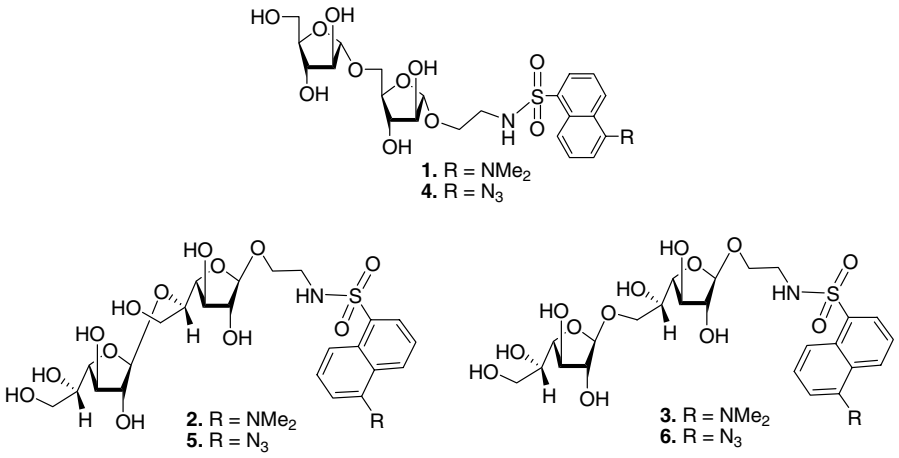


Chart 1. Fluorescent (**1**–**3**) and Photoaffinity (**4**–**6**) probes for glycosyltransferases in MTB.

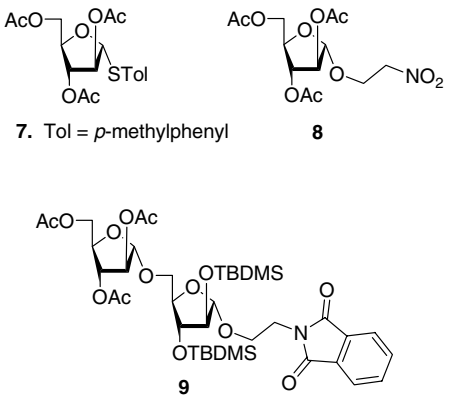


Figure 4.

yield. Unfortunately, deacetylation of **8** using several different reagents proved ineffective, and only a poor yield (48%) of deacetylated glycoside was achieved using K_2CO_3 at −20 °C. Alternatively, glycoside **7** was reacted with commercially available *N*-(2-hydroxyethyl)phthalimide to afford the desired ethylphthalimido α-glycoside. This product on deacetylation followed by blocking of the hydroxyl groups with *tert*-butyldimethylsilyl (TBDMS) groups and selective deprotection of the 5-OTBDMS group yielded the acceptor saccharide containing 5-OH group. This acceptor sugar was coupled with thioglycoside donor **7** resulting in the desired α 1 → 5-linked disaccharide **9**. The dephthaloylation of disaccharide **9** with hydrazine hydrate under several conditions failed to give the desired disaccharide possessing a free ethylamino disaccharide as single product, yielding an inseparable mixture of the blocked amino byproducts as a result of acetyl group migration.

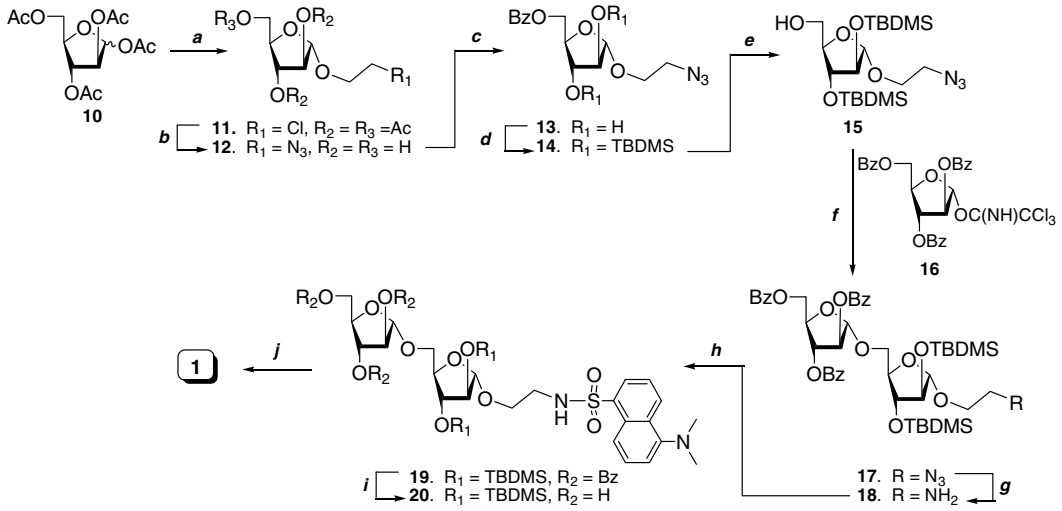
Finally, a chloroethyl group was introduced at the glycosidic linkage on the reducing end followed by displacement of chloride with azide to synthesize the desired α(1 → 5) linked disaccharide. This approach was also utilized to prepare the 1-*O*-ethylazido glycosides in the Gal*f* disaccharide probes. The fluorescent and photoaffinity disaccharides Ara*f*(α1 → 5)Ara*f*, Gal*f*(β1 → 5)Gal*f*, and Gal*f*(β1 → 6)Gal*f* probes (**1**–**6**) were readily synthesized as described (Scheme 1–5).

2.2. Synthesis of fluorescent probe for AraT's (Scheme 1)

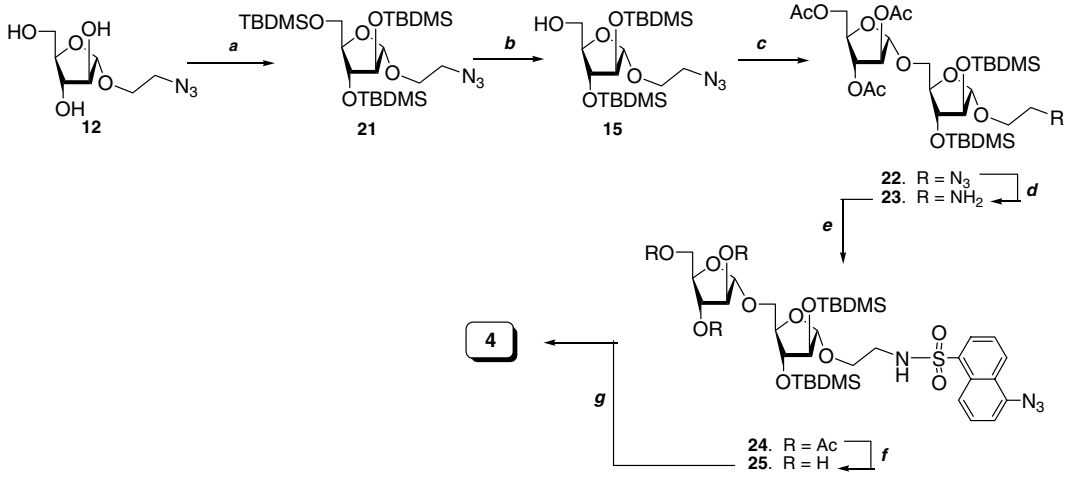
2.2.1. Synthesis of building blocks. Compound **10**, 1,2,3,5-tetra-*O*-acetyl-D-arabinofuranoside, was obtained from D-arabinose by reported methods.[17] Reaction of **10** with chloroethanol in the presence of $SnCl_4$ gave **11** as the pure α-isomer, anomeric proton as singlet at δ 5.48, after silica gel column chromatography.[18] The chloroethyl compound **11** was then heated with NaN_3 in dry DMF for 6 h followed by deacetylation with 7 N NH_3/MeOH to give **12** in an overall yield of 70% (two steps) after purification. The 5-position was selectively blocked using 1.0 equiv of benzoyl chloride in dry pyridine at −78 °C followed by warming to room temperature and leaving overnight. Standard workup and chromatography on Silica gel G gave compound **13** in moderate (58%) yield. Both the 2- and 3-positions were blocked with a TBDMS group to give glycoside **14** followed by treatment with 7 N NH_3/MeOH to obtain the acceptor **15** in high yield. The trichloroacetimidate donor **16** was synthesized as reported.[19]

2.2.2. Glycosylation and reduction. The trichloroacetimidate donor **16** and the acceptor azidoethyl-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (**15**) were reacted for 2 h in the presence of the Lewis acid promoter $BF_3{\cdot}Et_2O$ (1.0 equiv, dissolved in 2 mL of dry CH_2Cl_2). Additions were done at 0 °C, and the reaction was carried out under an inert atmosphere in dry CH_2Cl_2 at room temperature over powdered 4 Å molecular sieves. Column chromatography on Silica gel G afforded the pure disaccharide **17** in 66% yield. The azido group was reduced by heating with Ph_3P in a benzene/H_2O mixture followed by rapid purification via flash chromatography to afford compound **18**.

2.2.3. Incorporation of the fluorescent group. The relative instability of **18** necessitated immediate reaction with dansyl chloride in the presence of *N*-methylimidazole to produce the fluorescently labeled disaccharide **19** in



Scheme 1. Reagents and conditions: (a) $Cl(CH_2)_2OH$, $SnCl_4$, CH_3CN, rt, 85%; (b) NaN_3, DMF, 85 °C; 7 N NH_3/MeOH, rt, 70% in two steps; (c) BzCl, Py, −78 °C to rt, 58%; (d) TBDMSCl, DMF, imidazole, 85 °C, 81%; (e) 7 N NH_3/MeOH, rt, 93%; (f) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −20 °C, 66%; (g) Ph_3P, benzene, H_2O, 50 °C, 87%; (h) dansyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 95%; (i) 7 N NH_3–MeOH, rt, 93%; (j) $Et_4N^+F^-$, THF, rt, 97%.



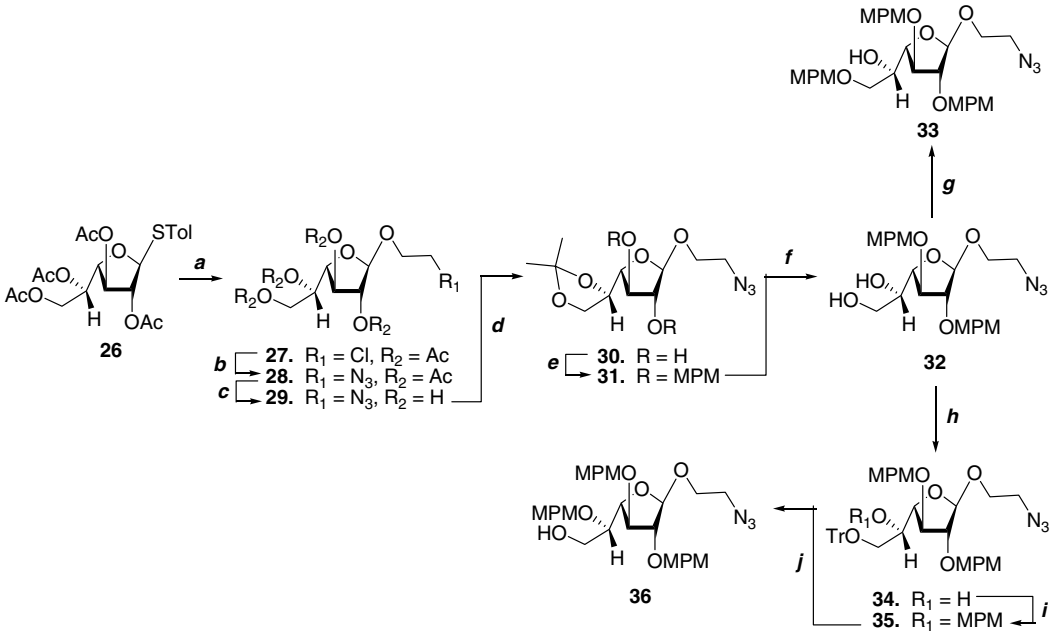
Scheme 2. Reagents and conditions: (a) TBDMSCl, DMF, imidazole, 60 °C, 90%; (b) TFA/water (1:1), 0 °C, 68%; (c) 7, NIS, $Sn(OTf)_2$, CH_2Cl_2, −20 °C, 91%; (d) HCO_2NH_4, MeOH, 5% Pd/C, rt, 87%; (e) 5-azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 81%; (f) 7 N NH_3–MeOH, rt, 87%; (g) $Et_4N^+F^-$, THF, rt, 80%.

high yield. Lastly, compound **20** obtained from **19** was deprotected to give the final fluorescent target **1**.

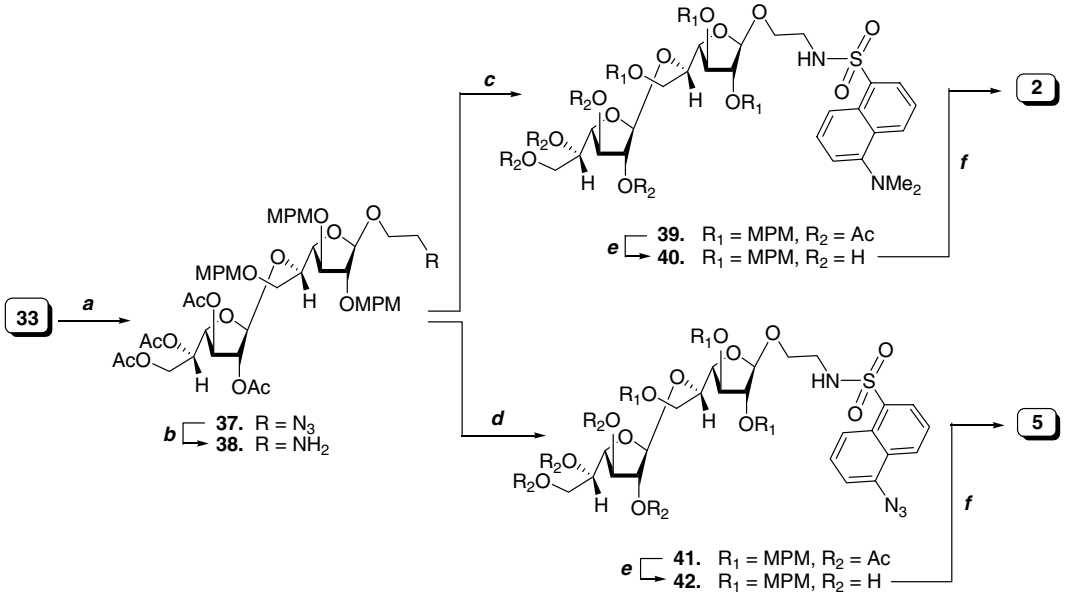
2.3. Synthesis of photoaffinity probe for AraTs (Scheme 2)

2.3.1. Synthesis of building blocks. The synthesis of the target probe **4** is represented in Scheme 2. A slightly different approach was adapted that was shorter and more efficient than the synthesis of **22** via selective protection of the 5-position with a benzoyl group as shown in Scheme 1. Compound **19** was first reacted with 3.5 equiv TBDMSCl in the presence of imidazole at 60 °C for two days affording compound **21** in 90% yield, followed by selective deprotection of only the 5-OTBDMS group using TFA/water (1:1) system at 0 °C. The preparation of compound **15** from compound **21** was successfully achieved in one step with a yield of 68% after column purification.

2.3.2. Glycosylation and reduction. The 1-thiocresyl-2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside donor **7**[20] and the acceptor azidoethyl-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside **15** were reacted for 2 h in the presence of the Lewis acid promoter $Sn(OTf)_2$ and activator *N*-iodosuccinimide. Addition of the reagents, and the subsequent reaction, was carried out at −20 °C under an argon atmosphere in dry CH_2Cl_2 over powdered 4 Å molecular sieves. Standard work-up followed by column chromatographic purification on Silica gel G afforded the pure disaccharide **22** in 91% yield. The *trans* glycosidation (α-linked disaccharide) was confirmed by the appearance of a singlet anomeric proton in ^{1}HNMR spectrum.[15] The azido



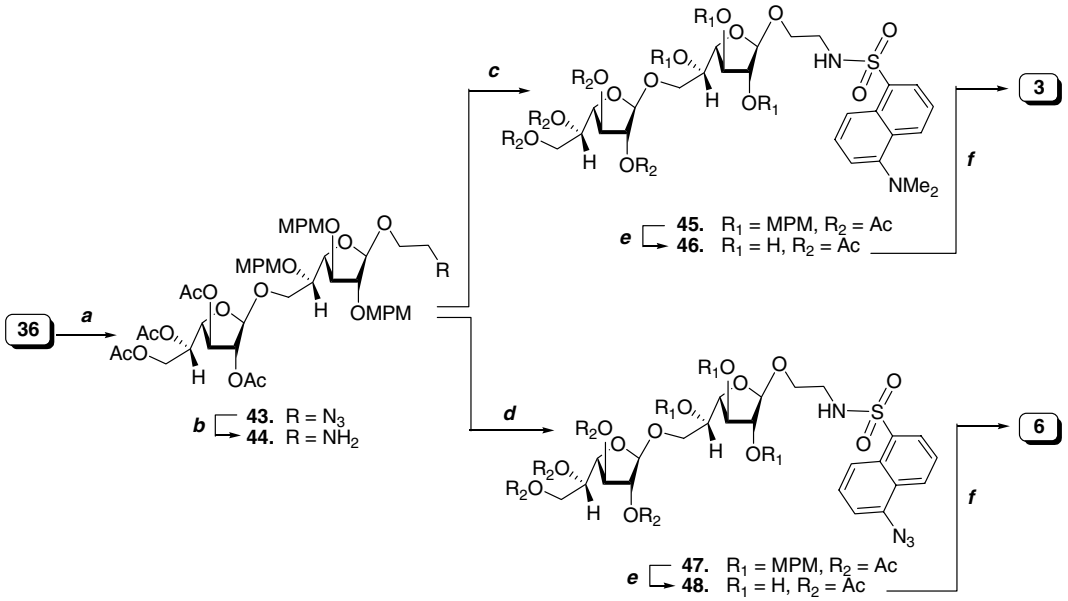
Scheme 3. Reagents and conditions: (a) $HOCH_2CH_2Cl$, NIS, $Sn(OTf)_2$, CH_2Cl_2, −20 °C to rt, 86%; (b) NaN_3, DMF, 70 °C, quant.; (c) 7 N NH_3–MeOH, rt, quant.; (d) 2,2-dimethoxypropane, camphor sulfonic acid, acetone, rt, 82%; (e) *p*-methoxybenzyl chloride (MPMCl), NaH, DMF, 0 °C to rt, 98%; (f) 60% aq AcOH, 60 °C, 77%; (g) Bu_2SnO, MPMCl, toluene, reflux to rt, 90%; (h) TrCl, DMAP, Py, rt–50 °C, 80%; (i) MPMCl, NaH, THF, 0 °C–rt, 86%; (j) 5% TFA in CH_3Cl, 0 °C, 70%.



Scheme 4. Reagents and conditions: (a) **26**, NIS, triflic acid, CH_2Cl_2, −20 °C, 66%; (b) Ph_3P, benzene, H_2O, 50 °C, 85%; (c) dansyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 77%; (d) azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 68%; (e) 7 N NH_3–MeOH, rt, **40**: quant., **42**: 92%; (f) $SnCl_4$, CH_2Cl_2, thiophenol, −78 to −20 °C, **2**: 33%, **5**: 78%.

group was reduced by reaction with ammonium formate ($H_2CO_2NH_4$) in dry methanol using 5% Pd/C as catalyst for 2 h at room temperature followed by rapid purification via flash chromatography to afford compound **23** in 87% yield. Again, the relative instability (probably a result of the acetyl blocking groups) of **23** necessitated immediate reaction of the free amine.

2.3.3. Incorporation of fluorescent group. Disaccharide **23** was reacted immediately with 5-azidonaphthalenesulfonyl chloride in the presence of *N*-methylimidazole at 0 °C over 4 h. The disaccharide **24** was obtained in good yield (81%). Lastly, compound **24** was deprotected via **25** to give the final target compound (5-azidonaphthalene-1-sulfonamidoethyl)-5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside **4**.[15]



Scheme 5. Reagents and conditions: (a) **26**, NIS, trflic acid, CH_2Cl_2, −20 °C, 43%; (b) Ph_3P, benzene, H_2O, 50 °C, 75%; (c) dansyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 60%; (d) azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 68%; (e) $SnCl_4$, CH_2Cl_2, thiophenol, −78 to −20 °C, **46**: 83%, **48**: 81%; (f) 7 N NH_3–MeOH, rt, **3**: quant., **6**: 80%.

2.4. Synthesis of probes for GT

2.4.1. Synthesis of building blocks. Thiocresyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside **26**, the building block for disaccharides Gal*f*(β 1 → 5)Gal*f* (**2**) and a Gal*f*(β 1 → 5)Gal*f* (**3**), was readily obtained from D-galactose by reported method.[10d,21] In a reaction sequence starting from thioglycoside **26**, both acceptors **33** and **36** were prepared possessing either a free 5-OH or 6-OH for coupling with thioglycoside donor **26** as represented in Scheme 3. Compound **26** was reacted with 1-chloroethanol in presence of NIS and Lewis acid promoter $Sn(OTf)_2$ in dichloromethane at −20 °C to produce **27** in 86% yield and α-glycosidation was confirmed by ^1H NMR spectra. Compound **27** on further reaction with NaN_3 in DMF at 70 °C gave the glycoside **28** possessing the ethylazido functionality. Compound **28** was deacetylated using 7 N NH_3/MeOH to give a quantitative yield of the deblocked glycoside **29**. The 5,6-hydroxyl groups in **29** were first blocked with an isopropylidene group using 2,2′-dimethoxypropane in the presence of a catalytic amount of (1*S*)-(+)-10-camphorsulfonic acid to yield **30**. Treatment of glycoside **30** with *p*-methoxybenzyl chloride and NaH afforded compound **31** in 98% yield. The ether protecting group was used due to the potential for ester group (benzoyl) migration between hydroxyl groups,[10d] and the *p*-methoxybenzyl protecting group was used specifically allowing ready deprotection of the disaccharides without affecting the azido group.

Saccharide **32** was regio-selectively benzylated via stannylated intermediates after reaction with dibutyltin oxide in boiling toluene and azeotropic removal of water.[10d] Evaporation gave the crude 5,6-*O*-stannylene acetal intermediate which was then selectively *p*-methoxybenzylated at the 6-position. Glycoside **33**, possessing a free 5-OH for coupling, was produced in 90% yield after silica gel flash column chromatography.

Glycoside **32** was selectively blocked using trityl chloride in pyridine at 50 °C to give the 6-tritylated derivative **34** in 72% yield. Glycoside **34** was then *p*-methoxybenzylated to give **35** in 86% yield. Finally, detritylation with 10% CF_3COOH in chloroform and further purification using column chromatography gave glycoside **36** possessing free 6-OH group in 70% yield.

2.4.2. Glycosylation and deprotection. Once the synthesis of acceptor glycosides **33** and **36** was achieved, disaccharides **37** and **43** were prepared using thioglycoside **26** as the donor, NIS as the promoter, and triflic acid as the Lewis acid as represented in Schemes 4 and 5, respectively.[10d]

2.4.2.1. Synthesis of Gal*f*(β 1 → 5)Gal*f* disaccharide. The synthesis of β1 → 5-linked Gal*f* disaccharide was carried out by coupling the acceptor glycoside **33** with thiocresyl glycoside donor **26** in dry dichloromethane at −20 °C in presence of promoter NIS and triflic acid to produce disaccharide **37** in 66% yield after column chromatography. β-Glycosidation was confirmed by the appearance of a singlet anomeric proton.[14] The azido group was reduced to the amine to produce disaccharide **38** by reduction with $H_2CO_2NH_4$ in the presence of 5% Pd/C in dry MeOH. Rapid purification gave pure disaccharide **38** in 92% yield as a colorless oil that was stored in a freezer (−20 °C) for further use to prepare the β1 → 5-linked Gal*f* probes.

2.4.2.2. Synthesis of Gal*f*(β 1 → 6)Gal*f* disaccharide. The synthesis of the β1 → 6-linked Gal*f* disaccharide was carried out by coupling glycoside **36** and the thio-

cresyl glycoside donor **26** in dry dichloromethane at −20 °C in the presence of promoter NIS and trifluoromethanesulfonic acid to produce disaccharide **43** in 65% yield after column chromatography. Again, the azido group in disaccharide **43** was reduced to an amino group using $H_2CO_2NH_4$ over 5% Pd/C in dry MeOH and purification gave disaccharide **44** in 98% yield. It was stored in the freezer (−20 °C) for later use.

2.5. Synthesis of fluorescent and photoaffinity probes for GT

2.5.1. Gal*f*(β1 → 5)Gal*f* fluorescent disaccharide (*2*) and photoaffinity probe (5). Disaccharide **2** was prepared by reaction of **38** with dansyl chloride at 0 °C in the presence of *N*-methylimidazole and dry CH_2Cl_2 to give disaccharide **39** in 77% yield after chromatography as shown in Scheme 4. Disaccharide **39** was deacetylated using 7N NH_3 in methanol to produce **40** in quantitative yield after purification. Treatment of **40** with ceric ammonium nitrate to remove the *p*-methoxybenzyl group in dichloromethane under standard conditions[22] was not effective. Addition of tin (IV) chloride and thiophenol to a cold dichloromethane solution of disaccharide **40** at −78 °C and further reaction at −20 °C overnight provided the deblocked disaccharide **2** in moderate 33% yield after purification. In a similar fashion, photoaffinity probe **5** was prepared by reaction of **38** with 5-azidonaphthalene sulfonyl chloride to produce disaccharide **41**. Deacetylation followed by debenzylation under conditions used to prepare **2** gave the target **5** in 78% yield.

2.5.2. Gal*f*(β1 → 6)Gal*f* fluorescent disaccharide (3) and photoaffinity probe (6). Synthesis of fluorescently labeled β1 → 6-linked Gal*f* disaccharide **3** and photoaffinity probe **6** was carried out in a similar fashion to the β1 → 5-linked Gal*f* disaccharides as represented in Scheme 5. Disaccharide **44** was reacted with dansyl chloride or 5-azidonaphthalene sulfonyl chloride and *N*-methylimidazole to produce **45** and **47**, respectively. These two disaccharides (**45** and **47**) were first debenzylated with $SnCl_4$ and thiophenol at sub zero temperatures to produce disaccharides **46** and **48** in 83% and 81% respective yields followed by deacetylation to give **3** (quantitative) and **6** (80%).

2.6. Structural elucidation

All mono- and disaccharides were characterized using NMR, MS, and CHN analysis. Whenever necessary, NOE, decoupling, and DEPT experiments were performed in order to confirm NMR assignments and stereochemistry at the anomeric center of all sugars. Coupling constant correlations were used to assign the individual protons. In Ara*f* glycosides the anomeric proton is seen as singlet or doublet with the $^3J_{1,2}$ value ⩽2.0 Hz, suggesting the formation of the α-stereocenter with the D-*arabino* configuration.[23] In Ara*f* disaccharides the H-1 anomeric protons of the reducing sugar (4.58–4.83 ppm) are generally seen at lower ppm as compared to non-reducing sugar H-1′ (4.87–5.38 ppm) with coupling constant $^3J_{1,2}$ and $^3J_{1',2'} \leqslant 2.0$ Hz (in most cases the coupling constant was found to be 0.0 Hz), suggesting *trans*-glycosylation and formation of α-linked disaccharides. Similar trends were seen in ^{13}C NMR spectra of disaccharides with C-1 present at higher ppm value (low field region) as compared to C-1′ in the range 110–105 ppm. The β-stereochemistry, *trans*-glycosylation, of all of the D-Gal*f* glycosides was evident by their appearance as a singlet or doublet with $^3J_{1,2}$ value ⩽2.0 Hz in the range of 4.98–5.08 ppm.[24] It is notable that H-1 appeared as ddd at 4.00 ppm with $^3J_{1,2}$ = 2.4 Hz in compound **29** (D_2O as solvent) with additional long-range coupling with H-3 and H-4 protons (*J* = values less ⩽1.0 Hz), and this observation may be attributable to conformational change in a polar solvent. ^1H NMR spectra of Gal*f* disaccharides showed a singlet or doublet due to anomeric H-1 proton in the range of 4.61–5.05 ppm and anomeric H-1′ in the range of 4.90–5.42 ppm with coupling constants $^3J_{1,2} \leqslant 2.0$ Hz. As with the Ara*f* disaccharides, the anomeric proton of the reducing sugar in Gal*f* disaccharides is seen at higher field as compared to a non-reducing sugar. In ^{13}C NMR spectra of Gal*f* glycosides the C-1 signal appeared between 108.6 and 105.4 ppm. However, in Gal*f* disaccharides the C-1 proton is seen between 105.8 and 109.9 ppm and C-1′ at higher field in a range between 105.2 and 109.5 ppm. Detailed NMR data (^1H and ^{13}C NMR) on all disaccharides (sugar portion) are presented in Tables 1–4 and the values confirmed the proposed configuration at the anomeric centers and the structures of synthesized disaccharides.

2.7. Glycosyltransferase activity of disaccharides

The effectiveness of these compounds as photoaffinity and fluorescent probes to study the mycobacterial glycosyltransferases (AraT's and GalT) requires that they act as enzyme substrates. As such, a cell-free assay system using the membrane fraction from *Mycobacterium smegmatis* was used to evaluate their acceptor activity.[10c,d,24]

2.7.1. Arabinosyltransferase activity. Based on the previous use of specific arabinose based neoglycolipid acceptors,[10] probe disaccharides **1** and **4** were assayed for acceptor activity. As expected, both compounds showed acceptor activity in assays performed in the presence of mycobacterial membrane fractions containing GT activity and resulted in [^{14}C]Ara*f* incorporation from DP-[^{14}C]A as the arabinose donor. TLC/autoradiography for the reaction products from **1** is shown in Figure 5. Data for probe **4** were described earlier.[15] Both probes demonstrated the enzymatic conversion of the disaccharides to their corresponding trisaccharide products. Different concentrations of **1** (2 mM maximum) and of **4** (8 mM maximum) were used for arabinosyltransferase activity. Calculation of kinetic constants revealed that probe **1** possessed a K_m value 0.68 mM, whereas probe **4** possessed a K_m value 3.07 mM. Based on these data, both probes are clearly substrates for the arabinosyltransferases. Compound **1** may be useful in the development of competition-based acceptor assay for the discovery of inhibitors of a crucial mycobacterial enzyme. Disaccharide **4**, on the other hand, may be useful

Table 1. ^{1}H NMR chemical shifts for Ara*f* disaccharides

Compound	Shifts δ (ppm), J (Hz)						
	Unit	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5a}$)	H-5a ($J_{5a,5b}$)	H-5b ($J_{4,5b}$)
17	Ara*f*	4.83 (d) (1.4)	4.06 (dd) (3.7)	3.99 (dd) (6.5)	4.08 (m) (5.2)	3.89 (dd) (11.2)	3.75 (dd) (3.7)
	Ara*f*′	5.38 (s) (0)	5.59 (d) (1.1)	5.56 (d) (4.8)	4.62 (ddd) (3.2)	4.84 (dd) (11.8)	4.62 (dd) (4.7)
18	Ara*f*	4.83 (d) (1.5)	4.04 (dd) (3.4)	3.99 (dd) (5.8)	4.07 (m) (5.4)	3.89 (dd) (11.0)	3.72 (dd) (4.3)
	Ara*f*′	5.37 (s) (0)	5.58 (s) (0)	5.57 (d) (7.0)	4.62 (ddd) (3.1)	4.84 (dd) (11.8)	4.69 (dd) (4.7)
19	Ara*f*	4.69 (d) (1.8)	4.04 (dd) (3.3)	3.91 (m) (nd)	3.98 (m) (4.6)	3.84 (dd) (10.9)	3.61 (dd) (3.5)
	Ara*f*′	5.32 (s) (0)	5.57 (s) (0)	5.56 (d) (4.0)	4.62 (ddd) (3.1)	4.83 (dd) (11.6)	4.68 (dd) (4.8)
20	Ara*f*	4.68 (d) (1.5)	3.92 (dd) (3.4)	3.77 (m) (nd)	3.99 (m) (nd)	3.77 (m) (10.8)	3.58 (dd) (3.2)
	Ara*f*′	5.07 (s) (0)	4.07 (d) (0.8)	3.99 (m) (2.3)	4.17 (dd) (2.4)	3.86 (dd) (11.8)	3.77 (m) (2.3)
22	Ara*f*	4.83 (d) (1.5)	4.05 (dd) (3.6)	3.97 (dd) (6.4)	4.01 (ddd) (4.6)	3.81 (dd) (11.2)	3.63 (dd) (3.8)
	Ara*f*′	5.11 (s) (0)	5.15 (d) (1.3)	4.97 (dd) (4.8)	4.29 (ddd) (3.0)	4.42 (dd) (11.3)	4.22 (dd) (5.6)
23	Ara*f*	4.82 (d) (1.4)	3.99 (m) (nd)	3.99 (m) (nd)	3.99 (m) (4.6)	3.82 (dd) (10.6)	3.60 (dd) (4.2)
	Ara*f*′	5.09 (s) (0)	5.14 (d) (1.5)	4.97 (dd) (4.7)	4.28 (ddd) (2.9)	4.42 (dd) (11.2)	4.22 (dd) (5.8)
24	Ara*f*	4.66 (d) (1.9)	3.91 (m) (nd)	3.91 (m) (nd)	3.91 (m) (4.5)	3.78 (dd) (10.8)	3.51 (m) (nd)
	Ara*f*′	5.07 (s) (0)	5.14 (d) (1.5)	4.99 (dd) (4.9)	4.29 (ddd) (3.1)	4.43 (dd) (11.4)	4.23(5.6)
25	Ara*f*	4.58 (d) (1.8)	3.80 (dd) (4.1)	3.93 (dd) (6.3)	3.71 (m) (nd)	3.71 (m) (nd)	3.62 (m) (nd)
	Ara*f*′	4.87 (d) (1.8)	3.99 (dd) (4.0)	3.86 (ddd) (6.6)	3.94 (ddd) (3.2)	3.74 (dd) (11.9)	3.63 (dd) (4.9)
1	Ara*f*	4.68 (d) (1.5)	3.92 (dd) (3.4)	3.77 (m) (nd)	3.99 (m) (nd)	3.77 (m) (10.7)	3.58 (dd) (3.2)
	Ara*f*′	5.07 (s) (0)	4.05 (d) (0.8)	3.99 (m) (4.5)	4.17 (dd) (2.3)	3.86 (dd) (11.8)	3.77 (m) (2.3)
4	Ara*f*	4.63 (d) (1.2)	3.79 (dd) (2.8)	3.84 (m) (5.8)	3.96 (ddd) (3.3)	3.73 (dd) (11.9)	3.63 (dd) (5.5)
	Ara*f*′	4.89 (d) (1.3)	3.96 (dd) (3.5)	3.84 (m) (5.8)	3.84 (m) (4.8)	3.76 (dd) (10.9)	3.52 (dd) (3.8)

Table 2. ^{13}C NMR chemical shifts for Ara*f* disaccharides

Compound	Unit	Shifts δ (ppm)				
		C-1	C-2	C-3	C-4	C-5
17	Ara*f*	108.5	82.1	79.1	84.1	66.5
	Ara*f*′	105.9	82.1	78.1	81.2	63.7
18	Ara*f*	108.3	87.4	79.2	83.8	66.8
	Ara*f*′	105.9	82.1	78.0	81.2	63.7
19	Ara*f*	108.3	82.6	78.6	83.5	66.6
	Ara*f*′	106.1	82.1	77.9	81.1	63.7
20	Ara*f*	108.5	83.5	79.1	82.5	65.8
	Ara*f*′	107.5	79.2	78.0	86.9	61.9
22	Ara*f*	108.5	84.1	78.8	82.0	66.4
	Ara*f*′	105.9	81.0	77.1	80.5	63.3
24	Ara*f*	108.4	82.6	78.6	83.4	66.5
	Ara*f*′	106.0	81.2	77.1	80.5	63.3
25	Ara*f*	109.6	85.3	79.9	83.2	67.4
	Ara*f*′	109.8	83.4	78.4	85.3	62.7
1	Ara*f*	107.7	82.5	76.9	81.2	61.5
	Ara*f*′	107.6	81.0	76.8	84.2	66.7
4	Ara*f*	109.6	83.1	79.0	85.9	67.5
	Ara*f*′	109.6	83.1	78.7	84.3	63.1

as a photoaffinity probe for labeling active site residues of the arabinosyltransferase.

2.7.2. Galactosyltransferase activity. All four Gal*f* probes **2**, **3**, **5**, and **6** were screened for acceptor activity.[10d] Assays were performed in the presence of membranes and the cell wall enzymatic fraction P60 and 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 all Gal*f* probes. Different concentrations of probes **2** and **3** in the range of 0–4 mM were used for galactosyltransferase activity. In the case of probes **5** and **6**, a concentration range of 0–6 mM was utilized. TLC/autoradiography (Figs. 6 and 8) demonstrated acceptor activity and the likely enzymatic conversion of all Gal*f* disaccharides to their corresponding trisaccharide products and incorporation of [^{14}C]Gal to the 5′-OH of **2** and **5** and the 6′-OH of **3** and **6**. Gal*f* disaccharides **3**, **4**, and **6** gave rise to a second, slower migrating band (Figs. 6 and 8) which, based on relative migration profiles, would be anticipated to be a tetrasaccharide product resulting from further elongation of the trisaccharide products at the 6′-OH or 5′-OH consistent with the alternating linkage pattern of AG. Calculation of kinetic constants (Figs. 7–9) revealed that **2** and **3** possessed K_m values 7.33 and 2.38 mM, respectively, whereas **5** and **6** possessed K_m values 2.77 and 1.98 mM, respectively. The acceptor data clearly suggested that disaccharide probes **2** and **3** can be used for the development of competition-based assays for inhibitor discovery, and that disaccharides **5** and **6** may be useful for probing the bi-functional galactosyltransferase.

2.8. Conclusion

In summary, we reported a detailed account of the syntheses of Ara*f* ($\alpha1 \rightarrow 5$) Ara*f*, Gal*f* ($\beta1 \rightarrow 5$) Gal*f*, and Gal*f* ($\beta1 \rightarrow 6$) Gal*f* disaccharides as potential fluorescent and photoaffinity probes (**1**–**6**). All probe disaccharides were found to be very stable at room temperature but were stored in the refrigerator at 4 °C in dark bottles when not in use. These disaccharides have shown acceptor activity in cell free assay systems for the target glycosyltransferases. Extensive developmental work utilizing these compounds is in progress and will be reported elsewhere. Competition-based assays are being developed to substitute a fluorescence-based assay for the reported assays based on radiolabeled decaprenolphosphoarabinose (^{14}C-DPA) for AraTs and UDP-[^{14}C]Gal*f* (used for *in situ* preparation of UDP-[^{14}C]Gal*p*) for GalT. Recently, we have reported the use of Gal*f* ($\beta1 \rightarrow 5$) Gal*f* and Gal*f* ($\beta1 \rightarrow 6$) Gal*f* disaccharides as potential

Table 3. ^{1}H NMR chemical shifts for Gal*f* disaccharides

Compound	Shifts δ (ppm), J (Hz)							
	Unit	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6a}$)	H-6a ($J_{6a,6b}$)	H-6b ($J_{5,6b}$)
37	Gal*f*	5.05 (s) (0)	4.01 (s) (0)	4.07 (m) (5.9)	4.00 (dd) (3.5)	4.07 (m) (3.6)	3.62 (dd) (10.2)	3.59 (dd) (3.6)
	Gal*f*′	5.24 (s) (0)	5.17 (d) (1.8)	4.96 (dd) (5.4)	4.35 (dd) (4.2)	5.32 (ddd) (3.6)	4.24 (dd) (12.0)	4.13 (dd) (7.8)
38	Gal*f*	5.04 (s) (1.2)[a]	3.98[a](dd) (3.0)	3.99 (m) (*n.d.*)	4.06 (m) (nd)	4.06 (m) (3.6)	3.62 (dd) (10.4)	3.59 (dd) (3.6)
	Gal*f*′	5.42 (s) (0)	5.17 (d) (1.8)	4.95 (dd) (4.8)	4.36 (dd) (4.2)	5.32 (ddd) (3.6)	4.24 (dd) (12.0)	4.14 (dd) (7.8)
39	Gal*f*	4.87 (s) (1.2)[a]	3.83[a](dd) (2.4)	3.97 (m) (*n.d.*)	3.97 (m) (nd)	3.97 (m) (7.2)	3.55 (dd) (10.4)	3.50 (dd) (3.6)
	Gal*f*′	5.39 (s) (0)	5.15 (d) (1.8)	4.95 (dd) (5.4)	4.35 (dd) (3.6)	5.31 (ddd) (3.6)	4.24 (dd) (12.0)	4.12 (dd) (7.8)
40	Gal*f*	4.88 (s) (0)	3.81 (d) (1.8)	4.06 (dd) (2.4)	4.01 (dd) (6.0)	3.96 (ddd) (4.2)	3.44 (dd) (nd)	3.44 (dd) (6.0)
	Gal*f*′	5.26 (s) (0)	4.01 (s) (0)	3.79 (m) (5.4)	3.98 (dd) (5.4)	3.79 (m) (6.6)	3.62 (dd) (11.4)	3.56 (dd) (nd)
41	Gal*f*	4.87 (d) (1.2)	3.79 (m) (nd)	3.98 (m) (nd)	3.98 (m) (nd)	3.98 (m) (6.1)	3.54 (dd) (10.7)	3.49 (dd) (4.5)
	Gal*f*′	5.37 (s) (0)	5.14 (d) (1.6)	4.95 (dd) (5.6)	4.34 (m) (3.6)	5.31 (ddd) (3.6)	4.25 (dd) (12.2)	4.12 (dd) (7.5)
42	Gal*f*	4.81 (s) (0)	3.93[c] (dd) (1.5)	4.09 (dd) (3.8)	4.17 (dd) (3.2)	3.97 (ddd) (4.9)	3.86 (dd) (12.2)	3.78 (dd) (5.8)
	Gal*f*′	5.05 (s) (0)	5.34 (d) (1.6)	4.06 (dd) (5.1)	4.45 (dd) (3.4)	5.39 (ddd) (4.4)	4.39 (dd) (11.9)	4.22 (dd) (6.9)
43	Gal*f*	5.04 (s) (1.8)	4.00[b] (dd) (3.6)	3.96 (dd) (7.2)	4.06 (dd) (3.6)	3.71 (ddd) (4.8)	3.82 (dd) (10.2)	3.61 (dd) (7.2)
	Gal*f*′	5.06 (d) (1.2)	5.07(dd) (2.4)	4.95 (dd) (5.4)	4.23 (dd) (3.6)	5.38 (ddd) (4.2)	4.32 (dd) (12.0)	4.17 (dd) (7.8)
44	Gal*f*	5.04 (s) (0)	3.95 (m) (nd)	3.95 (m) (6.3)	4.04 (dd) (3.0)	3.61 (m) (nd)	3.76 (m) (10.4)	3.45 (dd) (5.0)
	Gal*f*′	5.04 (s) (0.5)	5.07[c](dd) (1.8)	4.98 (dd) (5.6)	4.24 (dd) (4.0)	5.38 (ddd) (4.0)	4.32 (dd) (11.9)	4.17 (dd) (7.4)
45	Gal*f*	4.86 (s) (0)	3.81 (d) (3.0)	3.91 (dd) (6.6)	4.01 (dd) (3.0)	3.67 (ddd) (4.8)	3.79 (dd) (10.2)	3.56 (dd) (7.2)
	Gal*f*′	5.01 (s) (0)	5.07 (d) (1.8)	4.98 (dd) (5.4)	4.22 (dd) (3.6)	5.36 (ddd) (4.2)	4.30 (dd) (12.0)	4.17 (dd) (7.2)
46	Gal*f*	4.80 (s) (0)	3.88 (s) (0)	4.10 (d) (2.1)	4.01 (t) (2.1)	4.03 (ddd) (3.8)	3.81 (dd) (10.5)	3.58 (dd) (8.6)
	Gal*f*′	5.06 (s) (0)	5.04 (m) (nd)	5.04 (m) (5.8)	4.31 (dd) (3.6)	5.36 (ddd) (4.2)	4.37 (dd) (12.0)	4.20 (dd) (7.8)
47	Gal*f*	4.83 (s) (0)	3.77 (m) (3.0)	3.90 (dd) (6.6)	3.98 (dd) (3.1)	3.66 (ddd) (3.1)	3.77 (m) (nd)	3.56 (m) (6.6)
	Gal*f*′	5.01 (s) (0)	5.07 (d) (2.1)	4.97 (dd) (5.8)	4.30 (dd) (3.7)	5.37 (ddd) (3.7)	4.27 (dd) (11.8)	4.16 (dd) (7.4)
48	Gal*f*	4.83 (s) (0)	3.92 (u) (nd)	4.14 (u) (nd)	4.07 (u) (nd)	4.03 (u) (4.0)	3.80 (dd) (10.5)	3.58 (dd) (8.5)
	Gal*f*′	5.06 (s) (0)	5.04 (d) (2.4)	5.06 (dd) (5.9)	4.31 (dd) (3.7)	5.37 (ddd) (4.2)	4.37 (dd) (11.9)	4.20 (dd) (7.1)
2	Gal*f*	4.66 (d) (1.5)	3.82 (dd) (3.3)	4.04 (dd) (6.1)	3.91 (dd) (3.7)	3.86 (ddd) (6.6)	3.67 (dd) (11.8)	3.64 (dd) (5.2)
	Gal*f*′	5.16 (d) (0.6)	3.99 (m) (nd)	3.99 (m) (nd)	4.10 (u) (3.1)	3.72 (ddd) (6.5)	3.64 (dd) (11.2)	3.61 (dd) (6.6)
3	Gal*f*	4.67 (d) (1.5)	3.81 (dd) (3.1)	3.96[d] (ddd) (5.4)	3.82 (m) (nd)	3.82 (m) (4.6)	3.74 (dd) (10.2)	3.48 (dd) (7.0)
	Gal*f*′	4.91 (d) (1.6)	3.98 (dd) (3.3)	4.02[d] (ddd) (5.8)	3.99 (dd) (3.2)	3.72 (ddd) (5.7)	3.64 (dd) (11.2)	3.61 (dd) (6.9)
5	Gal*f*	4.61 (d) (1.6)	3.77 (dd) (3.3)	4.02 (dd) (5.9)	3.86 (dd) (nd)	3.84 (m) (6.4)	3.66 (dd) (11.8)	3.62 (m) (*n.d.*)
	Gal*f*′	5.15 (d) (1.5)	3.99 (m) (nd)	3.99 (m) (nd)	4.09 (m) (5.9)	3.71 (ddd) (3.1)	3.62 (m) (11.1)	3.60 (dd) (6.6)
6	Gal*f*	4.63 (d) (1.4)	3.76 (dd) (3.1)	3.95 (dd) (5.7)	3.77 (dd) (3.8)	3.81 (ddd) (5.0)	3.72 (dd) (10.4)	3.46 (dd) (7.2)
	Gal*f*′	4.90 (d) (1.4)	3.98 (dd) (3.3)	4.02 (dd) (5.9)	3.99 (dd) (3.2)	3.72 (dd) (5.6)	3.63 (dd) (11.2)	3.61 (dd) (6.9)

nd, not determined due to complexity of signal; u, unresolved signal.
[a] Showed $J_{1,2}$ = 1.2 Hz.
[b] Showed $J_{1,2}$ = 1.8 Hz.
[c] Showed $J_{1,2}$ = 0.5 Hz.
[d] Showed $J_{1,3}$ = 0.5 Hz.

photoaffinity probes to identify the galactosyltransferase (*glf*T) in MTB.[‡] Very recently the isolation and purification of *glf*T in milligram quantities has been reported by expression of the *Rv3808c* gene in *Escherichia coli* C41 (DE3) and purification of protein from the culture.[26] These probes may also be useful for labeling and identification of active site residues.

3. Experimental

3.1. Synthesis

3.1.1. General procedures. The reactions were performed under a dry argon atmosphere and reaction temperatures were measured externally. Anhydrous solvents were directly purchased and used as such in reactions. Whenever necessary, compounds and starting materials were dried by azeotropic removal of water with toluene under reduced pressure. The reactions were monitored by thin-layer chromatography (TLC) on precoated 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 sulfate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for work-up and chromatography were of reagent grade. Flash column chromatography was carried out on silica gel 60 (230–400 Mesh). ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. Specific ^{1}HNMR spectra were recorded at 600 MHz as required. The coupling constants (J) are reported in Hz and chemical shifts are in ppm (δ) relative to a residual solvent peak or an internal standard. FAB mass spectra were recorded either by adding NBA (3-nitrobenzyl alcohol) or LiCl and in some cases ESI-MS spectra were recorded on a BioTof-2 time-of-flight mass spectrometer. The presence of water, when presented in the analytical results, was confirmed by ^{1}H NMR as per journal requirements.

3.1.1.1. Chloroethyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside (11). To a dry CH_3CN (800 mL) solution of

[‡] 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. (communicated).

Table 4. ^{13}C NMR chemical shifts for Gal*f* disaccharides

Compound	Unit	Shifts δ (ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
37	Gal*f*	106.2	87.9	83.2	80.5	73.9	70.5
	Gal*f*′	105.1	80.9	76.5	80.4	69.4	63.0
38	Gal*f*	106.0	87.8	83.2	80.5	74.0	70.5
	Gal*f*′	105.1	80.9	76.5	80.4	69.3	63.0
39	Gal*f*	105.8	87.4	82.9	81.1	74.1	70.3
	Gal*f*′	105.2	81.0	76.4	80.3	69.3	63.0
40	Gal*f*	106.6	86.2	83.5	81.9	73.4	69.3
	Gal*f*′	105.3	87.4	78.7	78.6	70.9	63.8
41	Gal*f*	105.9	87.4	82.9	81.3	74.3	70.2
	Gal*f*′	105.2	81.1	76.5	80.3	69.4	63.1
42	Gal*f*	107.8	81.2	77.6	84.7	77.1	62.7
	Gal*f*′	105.6	80.1	76.2	81.8	69.2	61.6
43	Gal*f*	106.5	88.1	82.1	80.5	75.1	67.8
	Gal*f*′	105.7	81.1	76.5	80.1	69.3	62.7
44	Gal*f*	105.9	87.4	81.9	81.1	75.5	67.7
	Gal*f*′	105.7	81.2	76.5	80.0	69.3	62.7
45	Gal*f*	108.3	86.1	79.2	78.5	71.0	69.3
	Gal*f*′	106.3	81.8	76.0	79.9	69.1	62.6
46	Gal*f*	106.0	87.4	81.9	81.2	75.5	67.6
	Gal*f*′	105.7	81.1	76.5	80.1	69.3	62.8
47	Gal*f*	108.2	85.7	79.4	78.1	69.7	69.1
	Gal*f*′	106.2	79.9	76.1	81.7	69.2	62.7
2	Gal*f*	109.4	83.1	78.5	84.3	77.2	64.3
	Gal*f*′	109.2	82.6	78.8	85.0	72.3	62.8
3	Gal*f*	109.9	82.9	78.9	85.0	72.6	70.6
	Gal*f*′	109.5	82.8	78.8	85.6	71.1	64.5
5	Gal*f*	109.3	82.6	78.5	84.2	77.1	64.2
	Gal*f*′	109.1	83.1	78.7	84.9	72.2	62.7
6	Gal*f*	109.9	82.9	78.9	85.5	71.0	70.5
	Gal*f*′	109.4	82.8	78.8	85.0	72.5	64.4

2,3,5,6-tetra-*O*-acetyl-α-D-arabinofuranoside **10** (26.0 g, 81.69 mmol) was added $SnCl_4$ dropwise and the mixture was stirred for 30 min at room temperature. 1-Chloroethanol (7.5 mL) was added dropwise to the reaction which was stirred for another 1 h at room temperature. Celite (5.0 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 extracted with chloroform (2× 500 mL). The chloroform layer was again extracted with a cold-saturated solution of $NaHCO_3$ (2× 100 mL) followed by water (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and concentrated to an oil. Column chromatography ($CHCl_3$ 100%) afforded pure α-isomer **11** (22.1 g, 85%) as an oil. FABMS: *m/z* 525 $[M+H]^+$. Anal. Calcd for $C_{13}H_{19}ClO_8$: C, 46.14; H, 5.65. Found C, 46.09; H, 5.69. ^1H NMR (DMSO-d_6): δ 5.61 (d, 1H, $J = 4.4$ Hz, H-3), 5.48 (s, 1H, H-1), 5.41 (d, 1H, $J = 1.2$ Hz, H-2), 4.76 (dd, 1H, $J = 5.0$, 13.8 Hz, H-5a), 4.64 (dd, 1H, $J = 4.7$, 13.8 Hz, H-5b), 4.65 (m, 1H, H-4), 3.95 (m, 1H, OCH_2), 3.87 (m, 3H, OCH_2, CH_2Cl).

3.1.1.2. Azidoethyl α-D-arabinofuranoside (12). To a dry DMF (500 mL) solution of compound **11** (31.0 g, 91.52 mmol) was added NaN_3 (8.9 g, 137.28 mmol). The reaction mixture was heated at 85 °C for 6 h, left at rt for 2 days at which time 300 mL of acetone/ether (2:1) was added. It was filtered through Celite, and the solid was washed with acetone/ether mixture (20 mL). The combined filtrate was concentrated to an oil. 7 N NH_3/MeOH (200 mL) was added under inert atmosphere and the mixture was stirred overnight at rt. The reaction mixture was concentrated to an oil and purified by column chromatography using $CHCl_3$/MeOH (95:5) as the eluting solvent to yield compound **12** (14.4 g, 70%). FABMS: *m/z* 220 $[M+H]^+$. Anal. Calcd for $C_7H_{13}N_3O_5 \cdot 1.0\ H_2O$: C, 35.44; H, 6.37; N, 17.71. Found C, 35.39; H, 6.57; N, 17.89. ^1H NMR (DMSO-d_6): δ 5.36 (d, 1H, $J = 5.4$ Hz, 2-OH), 5.12

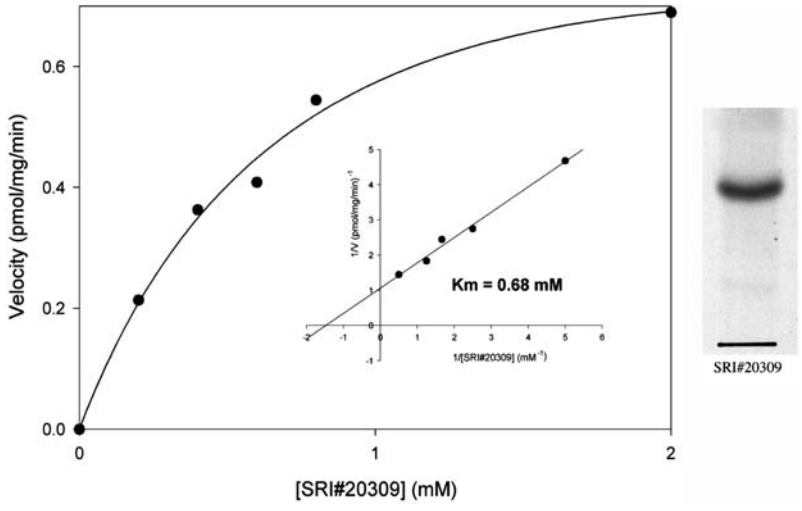


Figure 5. Kinetic analysis of acceptor 1 [SRI#20309—the inset shows the double reciprocal plot for 1 as a substrate for the arabinosyltransferase. The TLC autoradiogram shows the fluorescent trisaccharide reaction product mixture resulting from $\alpha(1 \rightarrow 5)$ and $\beta(1 \rightarrow 2)$ arabinofuranosyltransferase activities as reported earlier[25] through the inclusion of 1 at the highest concentration (2 mM), mycobacterial membranes and [^{14}C]-DPA. TLC/autoradiography was performed using chloroform/methanol/NH_4OH/H_2O (65:25:0.5:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 48 h.

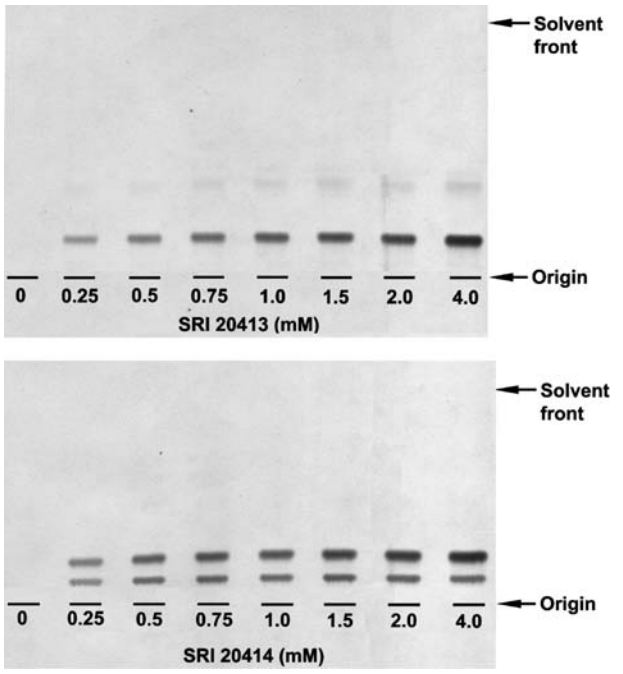


Figure 6. An autoradiogram of reaction products produced through the inclusion of disaccharides **2** (SRI 20413, upper panel) and **3** (SRI 20414, lower panel), mycobacterial membranes and UDP[^{14}C]Gal*f*. Lane 1, no acceptor; lane 2, 0.25 mM; lane 3, 0.5 mM; lane 4, 0.75 mM; lane 5, 1.0 mM; lane 6, 1.5 mM; lane 7, 2.0 mM; and lane 8, 4.0 mM. TLC/autoradiography was performed using $CHCl_3$/MeOH/NH_4OH/H_2O (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 3 days.

(d, 1H, J = 5.4 Hz, 3-OH), 4.77 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.72 (t, 1H, J = 5.6 Hz, 5-OH), 3.83 (dd, 1H, $J_{1,2}$ = 1.9 Hz, $J_{2,3}$ = 4.2 Hz, H-2), 3.78 (m, 2H, H-4, OCH_2), 3.68 (dd, 1H, $J_{2,3}$ = 4.2, $J_{3,4}$ = 7.1 Hz, H-3), 3.60 (dd, 1H, $J_{4,5a}$ = 3.0 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 3.57 (m, 1H, OCH_2), 3.46 (dd, 1H, $J_{4,5b}$ = 5.7 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5b), 3.44 (m, 2H, CH_2N_3).

3.1.1.3. Azidoethyl 5-*O*-benzoyl-α-D-arabinofuranoside (13). The compound **12** (14.4 g, 65.69 mmol) was azeotroped with pyridine and re-dissolved in 500 mL of dry pyridine. It was cooled to −78 °C and BzCl (7.6 mL, 65.69 mmol) was added dropwise. After complete addition, the reaction mixture was stirred overnight at rt. It was poured in ice-water mixture and extracted with $CHCl_3$ (200 mL). The organic layer was washed with water (2× 50 mL), dried over Na_2SO_4, and concentrated to oil. Column chromatography using $CHCl_3$/MeOH (98:2) as the eluting solvent gave compound **13** (12.4 g, 58%). FABMS (LiCl): *m*/*z* 330 $[M+Li]^+$. Anal. Calcd for $C_{14}H_{17}N_3O_6{\cdot}1.0\ H_2O$: C, 49.25; H, 5.61; N, 12.31. Found C, 49.29; H, 5.58; N, 12.29. ^{1}H NMR (DMSO-d_6): δ 7.98, 7.67, 7.54 (each m, Ph), 5.54 (d, 1H, J = 5.1 Hz, 2-OH), 5.43 (d, 1H, J = 5.3 Hz, 3-OH), 4.85 (d, 1H, $J_{1,2}$ = 2.0 Hz, H-1), 4.51 (dd, 1H, $J_{4,5a}$ = 2.9 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 4.31 (dd, 1H, $J_{4,5b}$ = 5.9 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5b), 4.07 (ddd, 1H, $J_{3,4}$ = 2.9 Hz, $J_{4,5a}$ = 2.9 Hz, $J_{4,5b}$ = 5.9 Hz, H-4), 3.87 (ddd, 1H, $J_{1,2}$ = 2.0 Hz, $J_{2,3}$ = 4.6 Hz, H-2), 3.78 (m, 2H, H-3, OCH_2), 3.59 (m, 1H, OCH_2), 3.40 (m, 2H, CH_2N_3).

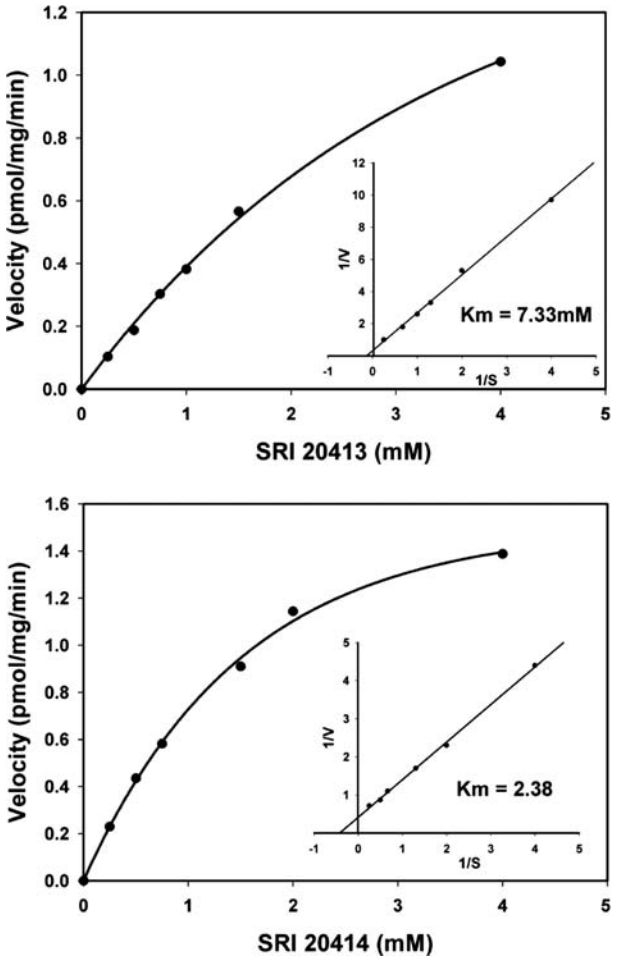


Figure 7. Kinetic analysis of acceptor disaccharides **2** (SRI 20413) and **4** (SRI 20414). The inset illustrates the double reciprocal plot for disaccharides as a substrate for the mycobacterial galactosyltransferase.

3.1.1.4. Azidoethyl 2,3-*O*-di-*tert*-butyldimethylsilyl-5-*O*-benzoyl-α-D-arabinofuranoside (14). Compound **13** (12.4 g, 38.39 mmol) was dissolved in dry DMF (500 mL) and to it were added TBDMSCl (21.5 g, 141 mmol) and imidazole (19.2 g, 282 mmol). The reaction mixture was heated at 60 °C for one day and concentrated. The residue was dissolved in $CHCl_3$ (40 mL) and washed with water (10 mL). Organic layer was dried over Na_2SO_4 and concentrated to syrup. Column chromatography (cyclohexane/EtOAc, 9:1) afforded compound **21** (17.1 g, 81%) as an oil. FABMS (NBA): *m*/*z* 558 $[M+H]^+$. Anal. Calcd for $C_{26}H_{45}N_3O_6Si_2$: C, 56.59; H, 8.23; N, 7.62. Found C, 56.58; H, 8.19; N, 7.70. ^{1}H NMR ($CDCl_3$): δ 8.07, 7.57, 7.44 (each m, Ph), 4.89 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 4.57 (dd, 1H, $J_{4,5a}$ = 3.3 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5a), 4.36 (dd, 1H, $J_{4,5b}$ = 5.7 Hz, $J_{5a,5b}$ = 11.9 Hz, H-5b), 4.23 (ddd, 1H, $J_{4,5a}$ = 3.3 Hz, $J_{4,5b}$ = 5.7 Hz, $J_{3,4}$ = 6.2 Hz, H-4), 4.10 (dd, 1H, $J_{1,2}$ = 1.3 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 4.04 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 6.2 Hz, H-3), 3.91 (m, 1H, OCH_2), 3.59 (m, 1H, OCH_2), 3.46 (m, 1H, CH_2N_3), 3.35 (m, 1H, CH_2N_3).

3.1.1.5. Azidoethyl 2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (15). *Method A:* Compound **14**

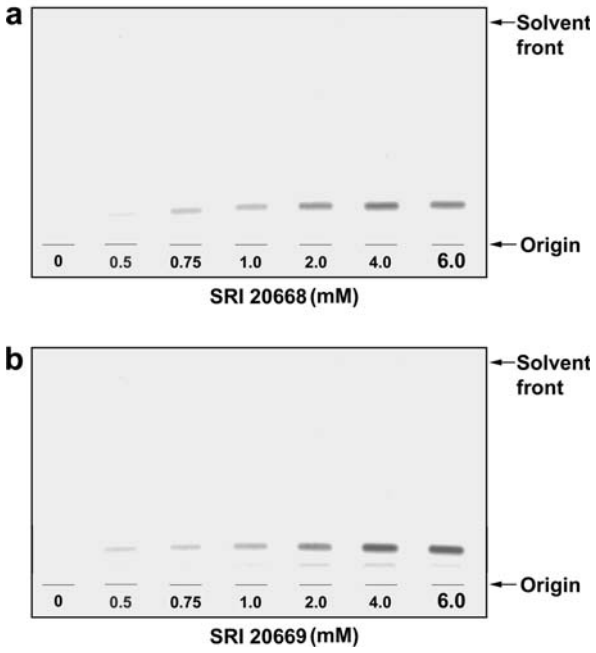


Figure 8. An autoradiogram of reaction products produced through the inclusion of disaccharides **5** (SRI 20668) and **6** (SRI 20669), mycobacterial membranes, and UDP[^{14}C]Gal*f*. Lane 1, no acceptor; lane 2, 0.5 mM; lane 3, 0.75 mM; lane 4, 1.0 mM; lane 5, 2.0 mM; lane 6, 4.0 mM; and lane 7, 6.0 mM. TLC/autoradiography was performed using $CHCl_3$/MeOH/NH_4OH/H_2O (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at −70 °C for 3 days.

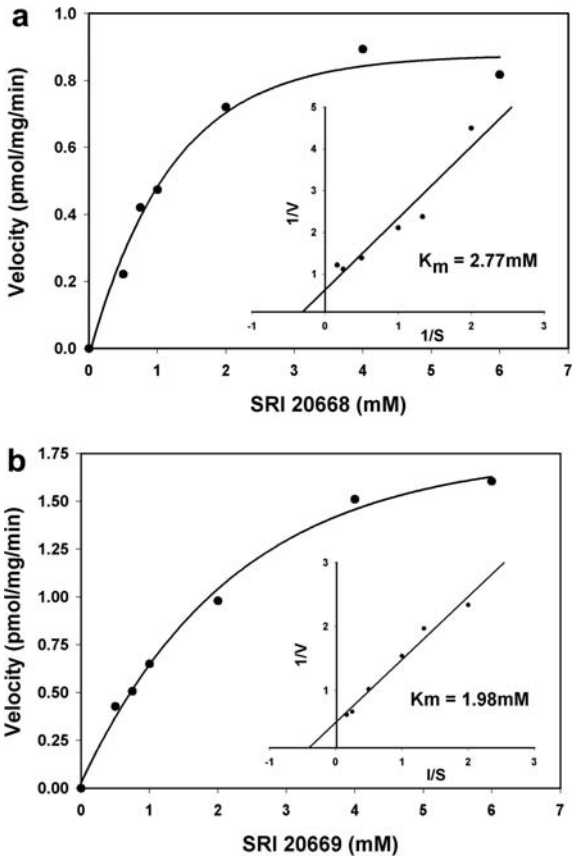


Figure 9. Kinetic analysis of acceptor disaccharides **5** (SRI 20668) and **6** (SRI 20669). The inset illustrates the double reciprocal plot for disaccharides as a substrate for the mycobacterial galactosyltransferase.

(17.0 g, 30.52 mmol) was dissolved in 7 N NH_3/MeOH (200 mL) under inert atmosphere and stirred overnight at rt. The reaction mixture was concentrated to an oil and subjected to column chromatography using cyclohexane/EtOAc (9:1) as the eluting solvent to give compound **15** (12.5 g, 93%) as a colorless oil.

Method B: To a THF (8.86 mL) solution of compound **21** (1.8 g, 3.20 mmol) was added TFA/H_2O (1:1, 17.5 mL) at 0 °C and the solution was stirred for 4 h. A saturated aqueous solution of $NaHCO_3$ (20 mL) was added and the mixture was diluted with $CHCl_3$ (50 mL). The organic layer was washed with 15 mL of deionized water and concentrated. Purification by column chromatography (cyclohexane/EtOAc, 3:1) afforded **15** (980 mg, 68%) as colorless oil. Spectral and analytical data reported earlier.[13]

3.1.1.6. Azidoethyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (17). A dry CH_2Cl_2 (20 mL) solution of **15** (1.00 g, 2.24 mmol) and **16** (1.63 g, 2.69 mmol) was cooled to −20 °C and stirred for 15 min. To it $BF_3{\cdot}Et_2O$ (0.34 mL, 2.69 mmol) was added dropwise and the reaction mixture was stirred for another 2 h at −20 °C. The reaction mixture was poured into a cold, saturated solution of $NaHCO_3$ (20 mL). The organic layer was washed with water (2× 25 mL), brine (20 mL) and dried over Na_2SO_4. After concentration and column chromatography (cyclohexane/EtOAc, 5:1), pure disaccharide **17** (1.2 g, 60%) was obtained as an oil. Spectral and analytical data have been reported.[14]

3.1.1.7. Aminoethyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (18). Compound **17** (1.0 g, 1.16 mmol) was dissolved in 50 mL of benzene, Ph_3P (608 mg, 2.32 mmol) was added, and the reaction mixture was heated to 50 °C. After 1 h, water (2.8 mL) was added and heating was continued for 5 h. The reaction mixture was concentrated and dissolved in $CHCl_3$, washed with water (2× 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, concentrated, and chromatographed ($CHCl_3$/MeOH, 95:5) to give **18** (845 mg, 87%) as a colorless oil. Spectral and analytical data have been reported.[13]

3.1.1.8. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (19). To a dry CH_2Cl_2 (25 mL) solution of **18** (805 mg, 0.93 mmol) was added *N*-methylimidazole and the mixture was cooled to 0 °C. To it 5-*N*,*N*-dimethylaminonaphthalene-sulfonyl chloride (378 mg, 1.40 mmol) was added and the reaction mixture was stirred at 0 °C for 3 h. The mixture was washed with water and brine, and the organic layer was dried over Na_2SO_4. Concen-

tration followed by column chromatography (cyclohexane/EtOAc, 5:1) gave **19** (970 mg, 95%) as a light yellow oil. Spectral and analytical data have been reported.[13]

3.1.1.9. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (20). Compound **19** (900 mg, 0.82 mmol) was debenzoylated with 7 N NH_3/MeOH (30 mL) as described for the preparation of **15**. Purification by column chromatography ($CHCl_3$/MeOH, 96:4) afforded **20** (567 mg, 88%) as a light yellow oil. FABMS (LiCl): *m/z* 793.5 $[M+Li]^+$. Anal. Calcd for $C_{36}H_{62}N_2O_{11}SSi_2$: C, 54.94; H, 7.95; N, 3.56. Found: C, 54.98; H, 7.92; N, 3.50. ^{1}H NMR ($CDCl_3$): non-sugar signals, δ 8.53 (d, 1H, J = 8.6 Hz, Ph), 8.29 (d, 1H, J = 8.7 Hz, Ph), 8.22 (dd, 1H, J = 1.2, 7.4 Hz, Ph), 7.53 (m, Ph), 7.18 (d, 1H, J = 7.6 Hz, Ph), 5.30 (m, 1H, NH, D_2O exchangeable), 3.50 (m, 2H, OCH_2), 3.25 (d, 1H, J = 11.4 Hz, 3′-OH), 3.08 (m, 2H, CH_2NH), 2.89 (s, 2H, 2× NCH_3), 0.87, 0.86 (s, 9H each 6× CH_3), 0.08 (s, 3H, CH_3), 0.06 (s, 6H, CH_3), 0.05 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): non-sugar signals, δ 151.9, 134.7, 130.4, 129.9, 129.6, 129.5, 128.3, 123.1, 118.9, 115.2 (Ph), 66.8 (OCH_2), 45.4 (NCH_3), 43.3 (CH_2NH), 25.7, 25.7 (CH_3), 17.83, 17.82 (C), −4.3, −4.61, −4.67, −4.8 (CH_3).

3.1.1.10. Azidoethyl 2,3,5-tri-*O*-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (21). Compound **12** (780 mg, 3.56 mmol) was silylated by reaction with TBDMSCl (1.84 mg, 12.5 mmol) and imidazole (1.5 mg, 21.36 mmol) in dry DMF (40 mL) as described for the preparation of **14**. Column chromatography (cyclohexane/EtOAc, 5:1) afforded **21** (1.80 g, 90%) as an oil. Spectral and analytical data have been reported.[14]

3.1.1.11. Azidoethyl 5-*O*-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (22). Alcohol **15** (980 g, 2.05 mmol), thioglycoside donor 1-deoxy-1-thiocresyl-2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside[19] **7** (942 mg, 2.46 mmol), and activated, powdered 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (30 mL) were cooled at 0 °C under argon atmosphere. The mixture was stirred for 15 min, and NIS (553 mg, 2.46 mmol) followed by $Sn(OTf)_2$ (86 mg, 0.21 mmol) were added to initiate coupling. The reaction mixture was stirred for 30 min at rt, and the reaction was quenched by addition of Et_3N (5 mL), diluted with CH_2Cl_2 (60 mL), and filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (20 mL), followed by washing with saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, the solvent was removed under vacuum, and the residue was purified by column chromatography (cyclohexane/EtOAc 3:1) to give pure disaccharide **22** (1.33 g, 91%) as an oil. ESI-MS: *m/z* 728.3233 $[M+Na]^+$, Calcd 728.3216 $[M+Na]^+$ for $C_{30}H_{55}N_3O_{12}Si_2$. ^{1}H NMR ($CDCl_3$): non-sugar signals, δ 3.88 (m, 1H, OCH_2), 3.57 (m, 1H, OCH_2), 3.44 (m, 1H, CH_2N_3), 3.34 (m, 1H, CH_2N_3), 2.10 (s, 6H, 2× $COCH_3$), 2.09 (s, 3H, CH_3), 0.99 (s, 9H, 3× CH_3), 0.88 (s, 9H, 3× CH_3), 0.102 (9H, s, 3× CH_3), 0.099 (s, 6H, 2× CH_3), 0.081 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): non-sugar signals, δ 170.6, 170.1, 169.4 ($COCH_3$), 66.4 (OCH_2), 50.8 (CH_2N_3), 25.8, 25.7 (C), 20.75, 20.72, 20.7 ($COCH_3$), 17.83, 17.79 (CH_3), −4.3, −4.7, −4.8, −4.9 (CH_3).

3.1.1.12. Aminoethyl 5-*O*-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (23). Disaccharide **22** (500 mg, 0.71 mmol) was dissolved in dry MeOH (10 mL) and 5% Pd/C was added (500 mg) under argon atmosphere. $H_2CO_2NH_4$ (179 mg, 2.83 mmol) was added and the reaction mixture was stirred at rt for 2 h. TLC showed complete conversion, and the reaction mixture was filtered through a short Celite pad and concentrated to syrup. The concentrate was dissolved in $CHCl_3$ (20 mL), washed with deionized water (10 mL), and dried over Na_2SO_4. Evaporation of the organic layer afforded the crude disaccharide **23** (420 mg) that was used as such to prepare **24**. ^{1}H NMR ($CDCl_3$): major non-sugar signals in crude sample, δ 3.71 (m, 1H, OCH_2), 3.43 (m, 1H, OCH_2), 2.86 (m, 2H, CH_2NH), 2.10 (s, 6H, CH_3), 2.09 (s, 3H, CH_3), 0.90 (s, 12H, CH_3), 0.88 (s, 12H, CH_3), 0.10, 0.09, 0.08 (CH_3's).

3.1.1.13. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (24). Crude 24 (420 mg, 0.62 mmol) was coupled with 5-azidonaphthalenesulfonyl chloride (248 mg, 0.93 mmol) in the presence of *N*-methylimidazole (102 mg, 1.24 mmol) using dry CH_2Cl_2 (50 mL) as solvent as described for the preparation of 19. Purification by column chromatography (cyclohexane/EtOAc, 3:1) afforded 24 (453 mg, 81%) as a light yellow oil (light sensitive). Spectral and analytical data have been reported.[14]

3.1.1.14. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(α-D-arabinofuranosyl)-2,3-*O*-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (25). Compound **24** (430 mg, 0.47 mmol) was deacetylated by 7 N NH_3/MeOH (6 mL) as described for the preparation of **20**. Column chromatography ($CHCl_3$/MeOH, 95:5) afforded pure **25** (322 mg, 87%) as a light sensitive, yellow oil. Spectral and analytical data have been reported.[14]

3.1.1.15. Chloroethyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (27). β-D-Galactofuranose pentaacetate (**26**)[21] (20.0 g, 51.28 mmol) was treated with $SnCl_4$ (6.0 mL, 51.28 mmol) and 1-chloroethanol (4.12 mL, 61.52 mmol) in dry CH_3CN (150 mL) as described for the preparation of **11**. Purification by column chromatography (cyclohexane/EtOAc, 2:1) yielded **27** (13.44 g, 64%) as an oil. FABMS (LiCl): *m/z* 417 $[M+Li]^+$. Anal. Calcd for $C_{16}H_{23}ClO_{10}$: C, 46.78; H, 5.64. Found: C, 46.69; H, 5.60. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.39 (ddd, 1H, $J_{4,5}$ = 3.7 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 7.0 Hz, H-5), 5.09 (dd, 1H, $J_{1,2}$ = 0.7 Hz, $J_{2,3}$ = 2.0 Hz, H-2), 5.08 (s, 1H, H-1), 5.01 (ddd, 1H, $J_{1,3}$ = 0.7 Hz, $J_{2,3}$ = 2.0 Hz, $J_{3,4}$ = 6.0 Hz, H-3), 4.34 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.33 (ddd, 1H, $J_{1,4}$ = 0.3 Hz, $J_{4,5}$ = 3.5 Hz, $J_{3,4}$ = 6.0 Hz, H-4), 4.21 (dd, 1H, $J_{5,6b}$ = 7.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 3.90 (m, 1H, OCH_2), 3.78 (m, 1H, OCH_2), 3.66 (m, 2H,

CH_2Cl), 2.14, 2.12, 2.10, 2.07 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 170.00, 169.95, 169.6 ($COCH_3$), 105.5 (C-1), 81.3 (C-2), 80.1 (C-4), 76.3 (C-3), 70.0 (C-5), 67.7 (OCH_2), 62.4 (C-6), 42.6 (CH_2Cl), 20.8, 20.72, 20.68, 20.63 (4× $COCH_3$).

3.1.1.16. Azidoethyl 2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (28). To a dry DMF (70 mL) solution of compound **27** (4.80 g, 11.66 mmol) was added NaN_3 (1.89 g, 29.2 mmol), and the reaction mixture was stirred at 70 °C overnight. The mixture was concentrated to near dryness and 100 mL of acetone/ether (2:1) was added and the mixture was stirred for 20 min at rt. It was filtered and concentrated to dryness. The resulting oil was re-dissolved in $CHCl_3$ (200 mL), washed with water (2× 40 mL), dried over Na_2SO_4, and concentrated. Column chromatography (cyclohexane/EtOAc, 2:1) gave compound **28** as pale yellow oil (4.88 g, quant.). FABMS (LiCl): *m/z* 424 $[M+Li]^+$. Anal. Calcd for $C_{16}H_{23}N_3O_{10}$: C, 46.04; H, 5.56; N, 10.07. Found: C, 46.06; H, 5.62; N, 9.99. ^1H NMR (300 MHz, $CDCl_3$): δ 5.41 (ddd, 1H, $J_{4,5}$ = 3.7 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 7.0 Hz, H-5), 5.08 (d, 1H, $J_{2,3}$ = 1.9 Hz, H-2), 5.07 (s, 1H, H-1), 5.02 (ddd, 1H, $J_{1,3}$ = 0.4 Hz, $J_{2,3}$ = 1.9 Hz, $J_{3,4}$ = 5.7 Hz, H-3), 4.34 (dd, 1H, $J_{5,6a}$ = 4.7 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6a), 4.32 (ddd, 1H, $J_{4,5}$ = 3.7 Hz, $J_{3,4}$ = 5.7 Hz, H-4), 4.23 (dd, 1H, $J_{5,6b}$ = 7.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b), 3.89 (ddd, 1H, J = 3.6, 6.9, 10.6 Hz, OCH_2), 3.66 (ddd, 1H, J = 3.4, 6.1, 10.6 Hz, OCH_2), 3.45 (ddd, 1H, J = 3.4, 6.9, 13.3 Hz, CH_2N_3), 3.36 (ddd, 1H, J = 3.6, 6.1, 13.3 Hz, CH_2N_3), 2.14, 2.12, 2.09, 2.07 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 169.9, 169.8, 169.4 ($COCH_3$), 105.4 (C-1), 81.1 (C-2), 80.2 (C-4), 76.2 (C-3), 69.1 (C-5), 66.5 (OCH_2), 62.3 (C-6), 50.3 (CH_2N_3), 20.6, 20.54, 20.48, 20.43 (4× $COCH_3$).

3.1.1.17. Azidoethyl β-D-galactofuranoside (29). Compound **28** (4.48 g, 10.7 mmol) was debenzoylated with 7 N NH_3/MeOH (10 mL) as described for the preparation of **20**. Purification by column chromatography ($CHCl_3$/MeOH, 5:1) afforded **29** (2.67 g, 98%) as an oil. FABMS (NBA): *m/z* 250 $[M+H]^+$. Anal. Calcd for $C_8H_{15}N_3O_6{\cdot}1.5\ H_2O$: C, 34.78; H, 6.57; N, 15.21. Found: C, 34.77; H, 6.59; N, 15.20. ^1H NMR (300 MHz, D_2O): δ 4.00 (ddd, 1H, $J_{1,2}$ = 2.4 Hz, $J_{1,3}$ = 0.6 Hz, $J_{1,4}$ = 0.7 Hz, H-1), 3.98 (m, 2H, H-2, H-3), 3.90 (ddd, 1H, $J_{1,4}$ = 0.7 Hz, $J_{3,4}$ = 6.0 Hz, $J_{4,5}$ = 4.0 Hz, H-4), 3.80 (m, 1H, OCH_2), 3.73 (ddd, 1H, $J_{4,5}$ = 4.0 Hz, $J_{5,6a}$ = 4.5 Hz, $J_{5,6b}$ = 7.4 Hz, H-5), 3.64 (m, 1H, OCH_2), 3.60 (dd, 1H, $J_{5,6a}$ = 4.5 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 3.55 (dd, 1H, $J_{5,6b}$ = 7.4 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6b), 3.42 (m, 2H, CH_2N_3). ^{13}C NMR (75 MHz, D_2O): δ 107.6 (C-1), 83.0 (C-4), 81.3 (C-2), 76.9 (C-3), 70.9 (C-5), 66.9 (OCH_2), 63.0 (C-6), 50.6 (CH_2N_3).

3.1.1.18. Azidoethyl 5,6-isopropyledine-β-D-galactofuranoside (30). To a solution of compound **29** (2.60 g, 10.4 mmol) in dry acetone (20 mL) were added 2,2′-dimethoxypropane (2.57 mL, 20.9 mmol) and (1*S*)-(+)-10-camphorsulfonic acid (242 mg, 1.04 mmol) at rt. After 1 h of stirring, the pH was adjusted to 7 by adding Et_3N. Concentration under vacuum gave the product which was dissolved in $CHCl_3$ (250 mL), washed with saturated $NaHCO_3$ (50 mL) and water (50 mL), dried over Na_2SO_4, and concentrated to an oil. Purification by column chromatography ($CHCl_3$/MeOH, 99:1) yielded **30** (2.46 g, 82%) as an oil. FABMS (NBA): *m/z* 290 $[M+H]^+$. Anal. Calcd for $C_{11}H_{19}N_3O_6{\cdot}0.5\ H_2O$: C, 44.29; H, 6.76; N, 14.09. Found: C, 44.29; H, 6.79; N, 14.01. ^1H NMR (300 MHz, $CDCl_3$): δ 5.08 (s, 1H, H-1), 4.37 (ddd, 1H, $J_{4,5}$ = 1.4 Hz, $J_{5,6a}$ = 6.5 Hz, $J_{5,6b}$ = 7.2 Hz, H-5), 4.14 (dd, 1H, $J_{3,4}$ = 1.8 Hz, $J_{4,5}$ = 1.4 Hz, H-4), 4.12 (dd, 1H, $J_{5,6a}$ = 6.5 Hz, $J_{6a,6b}$ = 8.3 Hz, H-6a), 4.05 (m, 3H, H-2, H-3, 3-OH), 4.02 (dd, 1H, $J_{5,6b}$ = 7.2 Hz, $J_{6a,6b}$ = 8.3 Hz, H-6b), 3.93 (m, 1H, OCH_2), 3.65 (m, 1H, OCH_2), 1.46 (m, 2H, CH_2N_3), 2.88 (d, 1H, J = 11.6 Hz, 2-OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 110.0 (C), 108.5 (C-1), 85.6 (C-4), 78.6 (C-3), 78.4 (C-2), 75.5 (C-5), 65.9 (C-6), 65.5 (OCH_2), 50.6 (CH_2N_3), 25.5, 25.9 (2× CH_3).

3.1.1.19. Azidoethyl 5,6-isopropyledine-2,3-di-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (31). To a dry DMF solution (50 mL) of compound **30** (1.50 g, 5.19 mmol) was added NaH (60% suspension in mineral oil −498 mg, 20.76 mmol) followed by *p*-methoxybenzyl chloride (2.81 mL, 20.76 mmol) dropwise with cooling at 0 °C. The reaction mixture was stirred at rt overnight. The reaction mixture was cooled to 0 °C and MeOH (23 mL) was added to the reaction. The mixture was evaporated to near dryness and re-dissolved in $CHCl_3$ (200 mL). It was washed with water (50 mL), dried over Na_2SO_4, and purified by column chromatography (cyclohexane/EtOAc, 5:1) to give **31** (2.69 g, 98%) as an oil. FABMS (LiCl): *m/z* 536 $[M+Li]^+$. Anal. Calcd for $C_{27}H_{35}N_3O_8$: C, 61.24; H, 6.66; N, 7.93. Found: C, 61.11; H, 6.49; N, 7.77. ^1H NMR (300 MHz, $CDCl_3$): δ 7.29, 7.18 (m, each 2H, Ph), 6.89 (m, 4H, Ph), 5.07 (s, 1H, H-1), 4.44 (m, 4H, 2× CH_2Ph), 4.14 (ddd, 1H, $J_{4,5}$ = 6.8 Hz, $J_{5,6a}$ = 6.9 Hz, $J_{5,6b}$ = 7.3 Hz, H-5), 4.04 (dd, 1H, $J_{1,2}$ = 1.0 Hz, $J_{2,3}$ = 2.9 Hz, H-2), 4.00 (dd, 1H, $J_{3,4}$ = 7.0 Hz, $J_{4,5}$ = 6.8 Hz, H-4), 3.94 (m, 1H, OCH_2), 3.83 (dd, 1H, $J_{5,6a}$ = 6.9 Hz, $J_{6a,6b}$ = 6.4 Hz, H-6a), 3.81, 3.80 (s, each 3H, 2× OCH_3), 3.77 (dd, 1H, $J_{5,6b}$ = 7.3 Hz, $J_{6a,6b}$ = 6.4 Hz, H-6a), 3.72 (dd, 1H, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 3.61 (m, 1H, OCH_2), 3.47 (m, 1H, CH_2N_3), 3.32 (m, 1H, CH_2N_3), 1.40, 1.35 (s, each 3H, 2× CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.4, 159.3 (2× C), 129.7 (CH), 129.3 (C), 113.8, 113.7 (2× CH), 109.6 (C), 106.4 (C-1), 87.5 (C-2), 83.5 (C-3), 82.0 (C-4), 76.5 (C-6), 71.8, 71.6 (2× CH_2Ph), 66.3 (OCH_2), 65.3 (C-6), 55.2, 55.2 (2× OCH_3), 50.5 (CH_2N_3), 26.4, 25.4 (2× CH_3).

3.1.1.20. Azidoethyl 2,3-di-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (32). A solution of compound **31** (2.50 g, 4.72 mmol) in 60% aqueous acetic acid (75 mL) was stirred for 4 h at 60 °C. The solution was concentrated in vacuum, coevaporated with diluted $CHCl_3$ (20 mL), and neutralized with saturated $NaHCO_3$ solution. The organic phase was washed with water (2× 50 mL), dried over Na_2SO_4, and purified by column chromatography ($CHCl_3$/MeOH, 95:5) to give **32**

(1.78 g, 77%) as an oil. FABMS: *m/z* 507 $[M+NH_4]^+$. Anal. Calcd for $C_{24}H_{31}N_3O_8{\cdot}0.5\ H_2O$: C, 57.82; H, 6.47; N, 8.43. Found: C, 57.85; H, 6.47; N, 8.39. ^1H NMR (300 MHz, $CDCl_3$): δ 7.27, 7.20 (each m, each 2H, Ph), 6.88 (m, 4H, Ph), 5.04 (s, 1H, H-1), 4.46 (m, 4H, 2× CH_2Ph), 4.08 (dd, 1H, $J_{3,4}$ = 6.8 Hz, $J_{4,5}$ = 3.9 Hz, H-4), 4.03 (dd, 1H, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 2.9 Hz, H-2), 4.00 (dd, 1H, $J_{2,3}$ = 2.9 Hz, $J_{3,4}$ = 6.8 Hz, H-3), 3.85 (m, 1H, OCH_2), 3.81, 3.79 (s, each 3H, 2× OCH_3), 3.73 (m, 1H, H-5), 3.65 (m, 2H, H_2-6), 3.59 (m, 1H, OCH_2), 3.46 (m, 1H, CH_2N_3), 3.33 (m, 1H, CH_2N_3), 2.51 (d, 1H, J = 7.0 Hz, 5-OH), 2.23 (dd, 1H, J = 5.1, 7.3 Hz, 6-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.4, 159.32 (2× C), 129.7, 129.5 (3× CH), 129.4, 129.1 (2× C), 113.8, 113.8 (2× CH), 106.6 (C-1), 87.1 (C-2), 83.0 (C-3), 82.2 (C-4), 71.9, 71.8 (2× CH$_2$Ph), 71.4 (C-5), 66.3 (OCH_2), 64.3 (C-6), 55.2, 55.2 (2× OCH_3), 50.5 (CH_2N_3).

3.1.1.21. Azidoethyl 2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (33). A toluene (150 mL) solution of compound **32** (749 mg, 1.53 mmol) and dibutyltin oxide (380 mg, 1.53 mmol) was refluxed overnight with azeotropic removal of water. The reaction mixture was cooled and evaporated to dryness. The residue was dissolved in dry DMF (10 mL) and anhydrous CsF (465 mg, 3.06 mmol) and *p*-methoxybenzyl chloride (0.45 mL, 3.06 mmol) were added. The resulting mixture was stirred overnight at rt under argon, concentrated under vacuum, re-dissolved in chloroform (150 mL), and washed with 10% aqueous KF solution and water (10 mL). The combined organic phase was dried, concentrated, and chromatographed (cyclohexane/EtOAc, 3:1) to afford pure **33** (840 mg, 90%) as an oil. FABMS (LiCl): *m/z* 616 $[M+Li]^+$. Anal. Calcd for $C_{32}H_{39}N_3O_9{\cdot}0.5\ H_2O$: C, 62.12; H, 6.52; N, 6.79. Found: C, 62.15; H, 6.53; N, 6.75. ^1H NMR (300 MHz, $CDCl_3$): δ 7.23 (m, 6H, Ph), 6.87 (m, 6H, Ph), 5.04 (s, 1H, H-1), 4.44 (m, 6H, 3× CH_2Ph), 4.08 (ddd, 1H, $J_{1,4}$ = 0.8 Hz, $J_{3,4}$ = 6.9 Hz, $J_{4,5}$ = 3.5 Hz, H-4), 4.01 (d, 1H, $J_{2,3}$ = 2.6 Hz, H-2), 4.00 (dd, 1H, $J_{2,3}$ = 2.6 Hz, $J_{3,4}$ = 6.9 Hz, H-3), 3.86 (m, 1H, H-5), 3.84-3.76 (m, 1H, OCH_2), 3.80, 3.795, 3.790 (each s, each 3H, 3× OCH_3), 3.57 (m, 1H, OCH_2), 3.48 (d, 2H, J = 6.0 Hz, H_2-6), 3.43 (m, 1H, CH_2N_3), 3.31 (m, 1H, CH_2N_3), 2.40 (d, 1H, J = 5.9 Hz, 5-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.3, 159.2, 159. 1 (3× C), 129.9, 129.6 (2× C), 129.6, 129.4, 129.2 (3× CH), 128.4 (C), 113.7, 113.67, 113.64 (3× CH), 106.4 (C-1), 87.2 (C-2), 82.8 (C-3), 81.5 (C-4), 72.9 (C-6), 71.7, 71.6, 71.1 (3× CH$_2$Ph), 69.9 (C-5), 66.2 (OCH_2), 55.15, 55.13, 55.12 (3× OCH_3), 50.4 (CH_2N_3).

3.1.1.22. Azidoethyl 2,3-di-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactofuranoside (34). Compound **32** (1.29 g, 2.64 mmol) was dissolved in dry pyridine (45 mL) and trityl chloride (1.47 g, 5.28 mmol) was added followed by DMAP (32 mg, 0.26 mmol). The reaction mixture was stirred overnight at 50 °C. The solution was concentrated to dryness and re-dissolved in $CHCl_3$ (200 mL). The $CHCl_3$ layer was washed with water (2× 50 mL), brine (2× 50 mL) and dried over Na_2SO_4. Concentration of the combined organic layers followed by column chromatography (cyclohexane/EtOAc, 2:1) gave pure **34** (1.54 g, 80%) as an oil. FABMS (LiCl): *m/z* 737 $[M+Li]^+$. Anal. Calcd for $C_{43}H_{45}N_3O_8$: C, 70.57; H, 6.20; N, 5.74. Found: C, 70.67; H, 6.23; N, 5.79. ^1H NMR (300 MHz, $CDCl_3$): δ 7.42 (m, 6H, Ph), 7.25 (m, 11H, Ph), 7.16 (m, 2H, Ph), 6.85 (m, 4H, Ph), 5.01 (s, 1H, H-1), 4.42 (m, 4H, CH_2Ph), 4.17 (m, 1H, H-4), 4.00 (d, 1H, $J_{2,3}$ = 2.4 Hz, H-2), 3.98 (dd, 1H, $J_{2,3}$ = 2.4 Hz, $J_{3,4}$ = 5.2 Hz, H-3), 3.84-3.76 (m, 2H, H-5, OCH_2), 3.80, 3.79 (s, each 3H, 2× OCH_3), 3.54 (m, 1H, OCH_2), 3.40 (m, 1H, CH_2N_3), 3.26 (m, 2H, H-6a, CH_2N_3), 3.17 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, $J_{6a,6b}$ = 9.4 Hz, H-6b), 2.32 (d, 1H, J = 7.1 Hz, 5-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.4, 159.2 (2× C), 143.8, 129.7 (2× C), 129.6, 129.4 (2× CH), 129.2 (C), 128.6, 127.7, 126.9 (3× CH), 113.8, 113.7 (2× CH), 106.5 (C-1), 87.2 (C-2), 86.6 (C), 83.0 (C-3), 81.7 (C-4), 71.9, 71.6 (2× CH$_2$Ph), 70.0 (C-5), 66.2 (OCH_2), 64.8 (C-6), 55.2 (OCH_3), 50.5 (CH_2N_3).

3.1.1.23. Azidoethyl 2,3,5-tri-*O*-*p*-methoxybenzyl-6-*O*-trityl-β-D-galactofuranoside (35). To a dry THF solution (50 mL) of compound **34** (1.54 g, 2.11 mmol) was added NaH (60% suspension in mineral oil −152 mg, 6.32 mmol) followed by *p*-methoxybenzyl chloride (0.85 mL, 6.32 mmol) dropwise with cooling at 0 °C. The reaction mixture was stirred at rt overnight. MeOH (20 mL) was added to the reaction, the mixture was evaporated to near dryness and re-dissolved in $CHCl_3$ (250 mL). It was washed with water (2× 50 mL), dried over Na_2SO_4, and purified by column chromatography (cyclohexane/EtOAc, 6:1) to give **35** (1.53 g, 86%) as an oil. FABMS (LiCl): *m/z* 857 $[M+Li]^+$. Anal. Calcd for $C_{51}H_{53}N_3O_9{\cdot}0.15\ H_2O$: C, 72.28; H, 6.18; N, 4.86. Found: C, 72.25; H, 6.22; N, 4.89. ^1H NMR (300 MHz, $CDCl_3$): δ 7.41 (m, 6H, Ph), 7.21 (m, 13H, Ph), 7.06 (m, 2H, Ph), 6.82 (m, 6H, Ph), 4.98 (d, 1H, $J_{1,2}$ = 1.4 Hz, H-1), 4.45 (m, 6H, 3× CH_2Ph), 4.10 (dd, 1H, $J_{3,4}$ = 7.6 Hz, $J_{4,5}$ = 3.1 Hz, H-4), 3.96 (dd, 1H, $J_{1,2}$ = 1.4 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 3.89 (dd, 1H, $J_{2,3}$ = 3.8 Hz, $J_{3,4}$ = 7.6 Hz, H-3), 3.80, 3.76, 3.75 (s, each 3H, OCH_3), 3.72 (m, 1H, OCH_2), 3.63 (ddd, 1H, $J_{4,5}$ = 3.1 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 5.9 Hz, H-5), 3.50 (m, 1H, OCH_2), 3.35 (m, 2H, H-6a, CH_2N_3), 3.22 (m, 2H, H-6b, CH_2N_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.3, 159.19, 159.17 (3× C), 143.9, 130.3, 129.93 (3× C), 129.89 (CH), 129.7 (C), 129.6, 129.5, 128.7, 127.7, 126.9, 113.7, 113.7, 113.6 (CH), 106.1 (C-1), 88.3 (C-2), 86.9 (C), 82.3 (C-3), 80.6 (C-4), 75.7 (C-5), 72.9, 71.7, 71.7 (3× CH$_2$Ph), 66.1 (OCH_2), 63.6 (C-6), 55.23, 55.20, 55.1 (3× OCH_3), 50.5 (CH_2N_3).

3.1.1.24. Azidoethyl 2,3,5-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (36). Compound **35** (1.40 g, 1.64 mmol) in $CHCl_3$ (25 mL) was chilled to 0 °C. 5% TFA/$CHCl_3$ (11 mL) was added dropwise over a period of 1.5 h at 0 °C. The reaction mixture was then stirred for another 5 h at 0 °C, neutralized with satd. $NaHCO_3$, and extracted with $CHCl_3$ (200 mL). The organic layer was washed with water (2× 50 mL), dried over Na_2SO_4, and concentrated. Purification by column chromatography (cyclohexane/EtOAc, 3:1) afforded **36** (700 mg, 70%) as an oil. FABMS (LiCl): *m/z* 616 $[M+Li]^+$. Anal.

Calcd for $C_{32}H_{39}N_3O_9$: C, 63.04; H, 6.45; N, 6.89. Found: C, 63.00; H, 6.42; N, 6.85. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (m, 6H, Ph), 6.86 (m, 6H, Ph), 5.06 (s, 1H, H-1), 4.44 (m, 6H, 3× CH_2Ph), 4.16 (dd, 1H, $J_{3,4}$ = 7.4 Hz, $J_{4,5}$ = 4.8 Hz, H-4), 4.02 (dd, 1H, $J_{1,2}$ = 1.1 Hz, $J_{2,3}$ = 3.3 Hz, H-2), 3.95 (dd, 1H, $J_{2,3}$ = 3.3 Hz, $J_{3,4}$ = 7.4 Hz, H-3), 3.87 (m, 1H, OCH_2), 3.81, 3.78, 3.77 (each s, each 3H, 3× OCH_3), 3.64 (m, 4H, H-5, H_2-6, OCH_2), 3.47 (m, 1H, CH_2N_3), 3.33 (m, 1H, CH_2N_3), 2.19 (dd, 1H, J = 4.8, 7.8 Hz, 6-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.3, 159.2, 159.1 (C), 130.2 (C), 129.6, 129.5 (2× CH), 129.3, 129.2 (2× C), 113.8, 113.7 (2× CH), 106.2 (C-1), 87.7 (C-2), 82.6 (C-3), 81.6 (C-4), 77.7 (C-5), 72.3, 71.8, 71.6 (3× CH$_2$Ph), 66.2 (OCH_2), 62.0 (C-6), 55.2, 55.1 (3× OCH_3), 50.5 (CH_2N_3).

3.1.1.25. Azidoethyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (37). Compound **33** (2.92 g, 4.78 mmol) was dissolved in dry CH_2Cl_2 (50 mL) and powdered 4 Å molecular sieves (~500 mg) were added under argon. The mixture was then cooled to −20 °C. The glycosylation donor **26** (3.02 g, 7.18 mmol) in 15 mL dry CH_2Cl_2 was added dropwise under argon. The mixture was stirred for 15 min, and NIS (2.16 g, 9.56 mmol) followed by triflic acid (4.0 μL, 0.48 mmol) were added. The reaction mixture was allowed to stir for 30 min at −20 °C, quenched by addition of Et_3N (5 mL), and filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (30 mL), followed by washing with saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, the solvent was removed under vacuum, and the residue was purified by column chromatography (cyclohexane/EtOAc, 2:1) to give **37** (3.72 g, 83%) as an oil. FABMS (LiCl): *m/z* 946.4 $[M+Li]^+$. Anal. Calcd for $C_{46}H_{57}N_3O_{18}\cdot 0.5\ H_2O$: C, 58.22; H, 6.16; N, 4.23. Found C, 58.19; H, 6.19; N, 4.27. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals, δ 7.22 (m, 6H, Ph), 6.86 (m, 6H, Ph), 4.50, 4.46, 4.42, 4.41, 4.39 (each d, 6H, J = 11.4 Hz, 3× CH_2Ph), 3.83 (m, 1H, OCH_2), 3.80, 3.794, 3.793 (each s, each 3H, 3× OCH_3), 3.58 (m, 1H, OCH_2), 3.44 (m, 1H, CH_2N_3), 3.32 (m, 1H, CH_2N_3). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.4, 170.03, 170.02, 169.3 (4× COCH$_3$), 159.4, 159.2, 159.1 (3× C), 129.8, 129.3, 129.1, 113.8, 113.7, 113.6 (12× CH), 72.9, 71.9, 71.7 (3× CH$_2$Ph), 66.3 (OCH_2), 55.2, 55.2 (3× OCH_3), 50.5 (CH_2N_3), 20.8, 20.7, 20.6, 20.5 (4× COCH$_3$).

3.1.1.26. Aminoethyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (38). Disaccharide **37** (3.00 g, 3.19 mmol) was dissolved in dry MeOH (50 mL) and 5% Pd/C was added (1.30 g) under argon atmosphere. $H_2CO_2NH_4$ (807 mg, 12.8 mmol) was added and the reaction mixture was stirred at rt for 4 h. TLC showed complete conversion, and the mixture was filtered through a short Celite pad and concentrated to a syrup. It was dissolved in $CHCl_3$ (100 mL), washed with deionized water (20 mL), and dried over Na_2SO_4. Concentration afforded the crude disaccharide and chromatography ($CHCl_3$/MeOH, 9:1) yielded **38** (2.69 g, 92%) as an oil. FABMS (NBA): *m/z* 914 $[M+H]^+$. Anal. Calcd for $C_{46}H_{59}NO_{18}\cdot 0.5\ H_2O$: C, 59.86; H, 6.55; N, 1.52. Found C, 59.90; H, 6.49; N, 1.48. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals, δ 7.21 (m, 12H, Ph), 6.86 (m, 6H, Ph), 4.48, 4.40 (m, each 6H, 3× CH_2Ph), 3.81 (s, 3H, OCH_3), 3.79 (s, 6H, 2× OCH_3), 3.69 (m, 1H, OCH_2), 3.46 (m, 1H, OCH_2), 2.87 (m, 2H, CH_2NH$_2$), 2.08, 2.06, 1.98, 1.97 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals δ 170.4, 169.9, 169.9, 169.2 (4× COCH$_3$), 159.3, 159.2, 159.0 (4× C), 130.0, 129.7, 129.3 (3× C), 129.6, 129.3, 129.0 (3× CH), 113.7 (2× CH), 113.6 (CH), 72.9 (CH$_2$Ph), 71.7 (2× CH$_2$Ph), 69.2 (OCH_2), 63.0 (C-6′), 55.2 (OCH_3), 55.1 (2× OCH_3), 41.9 (CH_2NH_2), 20.7, 20.6, 20.5, 20.4 (4× COCH$_3$).

3.1.1.27. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (39). Compound **38** (350 mg, 0.38 mmol) was mixed with *N*-methylimidazole (63 mg, 0.76 mmol) and dansyl chloride (155 mg, 0.57 mmol) in dry CH_2Cl_2 (40 mL) at 0 °C as described for the preparation of **18**. Purification by column chromatography (cyclohexane/EtOAc, 1:1) afforded the **39** (340 mg, 77%) as an oil. FABMS (NBA): *m/z* 1148 $[M+H]^+$. Anal. Calcd for $C_{58}H_{70}N_2O_{20}S\cdot 0.5\ H_2O$: C, 60.25; H, 6.19; N, 2.42. Found C, 60.19; H, 6.18; N, 2.41. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals δ 8.53 (d, 1H, J 8.4 Hz, Ph), 8.27 (d, 1H, J = 8.4 Hz, Ph), 8.23 (dd, 1H, J 0.6, 7.8 Hz, Ph), 7.50 (dd, 1H, J = 7.8, 8.4 Hz, Ph), 7.48 (dd, 1H, J = 7.2, 8.4 Hz, Ph), 7.19 (m, 6H, Ph), 7.12 (d, 1H, J = 7.2 Hz, Ph), 6.87 (d, 4H, J = 8.4 Hz, Ph), 6.83 (d, 2H, J = 8.4 Hz, Ph), 5.33 (m, 1H, NH), 4.39 (m, 6H, 3× CH_2Ph), 3.799, 3.795, 3.791 (each s, each 3H, 3× OCH_3), 3.59 (m, 1H, OCH_2), 3.40 (m, 1H, OCH_2), 3.07 (m, 2H, CH_2NH), 2.86 (s, 6H, $N(CH_3)_2$), 2.08, 2.06, 1.98, 1.94 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals δ 170.5, 169.9, 169.2 (4× COCH$_3$), 159.4, 159.3, 159.0 (C), 151.9 (C) 134.8 (C), 130.3 (CH), 130.1 129.8, 129.7 (C), 129.6, 129.5, 129.4 (CH), 129.2 (C), 129.1, 128.3, 123.1, 118.7, 115.1, 113.8, 113.6 (CH), 72.9 (CH$_2$Ph), 71.7 (2× CH$_2$Ph), 65.7 (OCH_2), 55.2, 55.2, 55.1 (3× OCH_3), 45.3 ($N(CH_3)_2$), 43.0 (CH_2NH), 20.8, 20.7, 20.6, 20.5 (4× COCH$_3$).

3.1.1.28. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(β-D-galactofuranosyl)-2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (40). Disaccharide **39** (300 mg, 0.26 mmol) in dry methanol (5 mL) was treated with 7 N NH_3/MeOH (10 mL) overnight at rt. Concentration under vacuum followed by column chromatography ($CHCl_3$/MeOH, 9:1) yielded **40** (285 mg, 98%) as an oil. FABMS (NBA): *m/z* 980 $[M+H]^+$. Anal. Calcd for $C_{50}H_{62}N_2O_{16}S\cdot 1.0\ H_2O$: C, 60.23; H, 6.47; N, 2.81. Found C, 60.19; H, 6.43; N, 2.84. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals δ 8.51 (d, 1H, J 8.4 Hz, Ph), 8.25 (d, 1H, J = 9.0 Hz, Ph), 8.19 (dd, 1H, J 1.2, 8.4 Hz, Ph), 7.48 (m, 1H, Ph), 7.46 (m, 1H, Ph), 7.18 (m, 4H, Ph), 7.13 (dd, 2H, J = 2.4, 10.8 Hz, Ph), 7.11 (d, 1H, J = 7.2 Hz, Ph), 6.85 (m, 6H, Ph), 4.35

(m, 6H, 3× CH_2Ph), 3.79, 3.78, 3.76 (s, each 3H, 3× OCH_3), 3.56 (m, 2H, H-6′b, OCH_2), 3.36 (m, 1H, OCH_2), 3.03 (m, 2H, CH_2NH), 2.85 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals δ 159.5, 159.3, 159.2 (3× C), 151.8 (C), 134.7 (C), 130.3, 129.8 (CH), 129.7 (C), 129.6 (CH), 129.5 (C), 129.3 (CH), 129.2, 128.9 (C), 128.3, 123.1, 118.8, 115.1, 113.9, 113.8, 113.7 (10× CH), 73.0, 71.7, 71.6 (3× *C*H_2Ph), 65.3 (OCH_2), 55.3, 55.22, 55.20 (3× OCH_3), 45.3 ($N(CH_3)_2$), 42.8 (CH_2NH).

3.1.1.29. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,6-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (41). Compound **38** (1.70 g, 1.86 mmol) was mixed with *N*-methylimidazole (0.3 mL, 3.72 mmol) and 1-azido-5-naphthalenesulfonyl chloride (747 mg, 2.79 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C as described for the preparation of **18**. Purification by column chromatography (cyclohexane/EtOAc, 1:1) afforded the **41** (1.22 g, 68%) as an oil. ESI-MS: *m*/*z* 1167.37 $[M+Na]^+$. Anal. Calcd for $C_{56}H_{64}N_4O_{20}S{\cdot}0.5\ H_2O$: C, 58.28; H, 5.68; N, 4.85. Found C, 58.27; H, 5.59; N, 4.80. ^{1}H NMR (300 MHz, $CDCl_3$): non-sugar signals, δ 8.38 (m, 2H, Ph), 8.27 (dd, 1H, J = 1.2, 8.4 Hz, Ph), 7.55 (dd, 1H, J = 3.7, 7.6 Hz, Ph), 7.52 (dd, 1H, J = 3.4, 7.4 Hz, Ph), 7.26 (dd, 1H, J = 0.7, 15.5 Hz, Ph), 7.19 (m, 6H, Ph), 6.85 (m, 6H, Ph), 5.39 (t, 1H, J = 5.2 Hz, NH), 4.38 (m, 3× CH_2Ph), 3.79 (m, 3× OCH_3), 3.59 (m, 1H, OCH_2), 3.40 (m, 1H, OCH_2), 3.07 (m, 2H, CH_2NH), 2.08, 2.06, 1.98, 1.94 (s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals δ 170.6, 170.0, 169.4 (4× *C*OCH_3), 159.5, 159.4, 159.1 (C), 137.6 (C) 134.9 (C), 130.3 (CH), 130.1 129.6 (C), 129.6, 129.4 (CH), 129.2 (C), 129.2, 128.5, 128.2 (CH), 127.2 (C), 124.3, 121.0, 114.8, 113.9, 113.7 (CH), 73.0, 71.8, 71.7 (3× *C*H_2Ph), 65.7 (OCH_2), 55.3, 55.2 (3× OCH_3), 43.2 (CH_2NH), 20.8, 20.7, 20.6, 20.5 (4× CO*C*H_3).

3.1.1.30. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-β-D-galactofuranoside (42). Disaccharide **41** (1.17 g, 1.02 mmol) was dissolved in 110 mL of CH_2Cl_2/H_2O (10:1) and DDQ (1.16 g, 5.1 mmol) was added at rt. The reaction mixture was stirred for 4 h, dried over Na_2SO_4, and concentrated to syrup. Purification over silica gel using $CHCl_3$/MeOH (95:5) afforded pure disaccharide **42** (734 mg, 92%). ESI-MS: *m*/*z* 785.20 $[M+H]^+$. Anal. Calcd for $C_{32}H_{40}N_4O_{17}S{\cdot}1.5\ H_2O$: C, 47.35; H, 5.34; N, 6.90. Found C, 47.29; H, 5.33; N, 6.87. ^{1}H NMR (300 MHz, $CDCl_3$): non-sugar signals δ 8.41 (ddd, 1H, J = 0.7, 0.8, 8.8 Hz, Ph), 8.39 (ddd, 1H, J = 0.8, 1.2, 8.6 Hz, Ph), 8.27 (dd, 1H, J = 1.2, 7.4 Hz, Ph), 7.64 (dd, 1H, J = 7.6, 8.8 Hz, Ph), 7.54 (dd, 1H, J = 7.4, 8.6 Hz, Ph), 7.35 (dd, 1H, J = 0.7, 7.6 Hz, Ph), 5.91 (t, 1H, J = 5.9 Hz, NH), 3.69 (m, 1H, OCH_2), 3.44 (m, 1H, OCH_2), 3.28 (d, 1H, J = 6.5 Hz, 3-OH), 3.18 (d, 1H, J = 9.3 Hz, 2-OH), 3.09 (dd, 2H, J = 5.1, 10.4 Hz, CH_2NH), 2.87 (br s, 1H, 6-OH), 2.14, 2.13 (each s, each 3H, OCH_3), 2.08 (s, 6H, 2× OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 171.2, 170.4, 170.2 (4× *C*OCH_3), 137.6, 134.7 (C), 130.2 (CH), 129.1 (C), 128.5, 128.1 (CH), 127.1 (C), 124.3, 121.0, 114.8 (CH), 66.0 (OCH_2), 42.9 (CH_2NH), 20.8, 20.7, 20.5 (OCH_3).

3.1.1.31. Azidoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (43). Glycosylation was carried out by the reaction of acceptor **36** (2.20 g, 3.61 mmol) and donor **26** (2.30 g, 5.41 mmol) in the presence of NIS (1.60 g, 7.22 mmol) and triflic acid (30 μL, 0.36 mmol) in dry CH_2Cl_2 (60 mL) −20 °C as described for **37**. Purification by column chromatography (cyclohexane/EtOAc, 2:1) yielded disaccharide **43** (2.35 g, 69%) as an oil. FABMS (LiCl): *m*/*z* 946 $[M+Li]^+$. Anal. Calcd for $C_{46}H_{57}N_3O_{18}$: C, 58.78; H, 6.11; N, 4.47. Found C, 58.76; H, 6.08; N, 4.43. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals, δ 7.27 (dd, 2H, J = 0.3, 9.0 Hz, Ph), 7.22 (dd, 2H, J 0.3 Hz, 9.0 Hz, Ph), 7.13 (dd, 2H, J = 0.3, 9.0 Hz, Ph), 6.89 (dd, 2H, J = 0.3, 8.4 Hz, Ph), 6.82 (dd, 2H, J = 0.3, 9.0 Hz, Ph), 6.81 (dd, 2H, J = 0.3, 9.0 Hz, Ph), 4.60, 4.53, 4.44, 4.42, 4.38, 4.21 (each d, each 1H, J = 11.4 Hz, 3× CH_2Ph), 3.85 (m, 1H, OCH_2), 3.81, 3.764, 3.757 (s, each 3H, 3× OCH_3), 3.58 (m, 1H, OCH_2), 3.44 (m, 1H, CH_2N_3), 3.34 (m, 1H, CH_2N_3), 2.12, 2.10, 2.05, 2.03 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.5, 169.9, 169.5 (4× *C*OCH_3), 159.3, 159.22, 159.19 (3× C), 130.2 (C), 129.8 (CH), 129.7 (C), 129.6 (CH), 129.5 (C), 113.7, 113.67, 113.61 (16× CH), 73.0, 71.7, 71.6 (3× *C*H_2Ph), 66.5 (OCH_2), 55.2, 55.20, 55.17 (3× OCH_3), 50.5 (CH_2N_3), 20.8, 20.7, 20.6, 20.6 (4× CO*C*H_3).

3.1.1.32. Aminoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (44). Disaccharide **43** (2.35 g, 2.50 mmol) was dissolved in dry MeOH (50 mL) and 5% Pd/C was added (2.0 g) under argon atmosphere. $H_2CO_2NH_4$ (630 mg, 10.0 mmol) was added and the reaction mixture was stirred at rt for 4 h. TLC showed complete conversion, and the mixture was filtered through a short Celite pad and concentrated to syrup. It was dissolved in $CHCl_3$ (100 mL), washed with deionized water (20 mL), and dried over Na_2SO_4. Concentration afforded the crude disaccharide. Column chromatography ($CHCl_3$/MeOH, 9:1) gave **44** (2.20 g, 98%) as an oil. FABMS (NBA): *m*/*z* 914 $[M+H]^+$. Anal. Calcd for $C_{46}H_{59}NO_{18}$: C, 60.45; H, 6.51; N, 1.53. Found C, 60.48; H, 6.47; N, 1.54. ^{1}H NMR (600 MHz, $CDCl_3$): non-sugar signals δ 7.26, 7.23, 7.13 (m, each 2H, Ph), 6.88 (m, 2H, Ph), 6.82 (m, 4H, Ph), 4.61 (d, 1H, J = 11.5 Hz, CH_2Ph), 4.51 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.43, 4.42 (each d, 1H, J = 11.5 Hz, CH_2Ph), 4.38 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.22 (d, 1H, J = 11.5 Hz, CH_2Ph), 3.81, 3.77, 3.76 (s, each 3H, 3× OCH_3), 3.81-3.70 (m, 3H, OCH_2), 2.87 (t, 2H, J = 5.3 Hz, CH_2NH_2), 2.12, 2.10, 2.05, 2.03 (s, each 3H, 4× $COCH_3$).

3.1.1.33. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (45). Coupling of **44** (240 mg, 0.26 mmol) with dansyl chloride (106 mg, 0.40 mmol) was carried out in the presence of *N*-methylimidazole (43 mg, 0.52 mmol) in

dry CH_2Cl_2 (30 mL) at 0 °C as described for **39**. Purification by column chromatography (cyclohexane/EtOAc, 1:1) afforded **45** (180 mg, 60%) as an oil. FABMS (NBA): *m/z* 1148 $[M+H]^+$. Anal. Calcd for $C_{58}H_{70}N_2O_{20}S$: C, 60.72; H, 6.15; N, 2.44. Found C, 60.69; H, 6.13; N, 2.39. ^1H NMR (600 MHz, $CDCl_3$): non-sugar signals, δ 8.52 (d, 1H, J = 8.4 Hz, Ph), 8.27 (d, 1H, J = 8.4 Hz, Ph), 8.22 (dd, 1H, J = 1.2, 7.2 Hz, Ph), 7.31 (m, 2H, Ph), 7.21 (m, 5H, Ph), 7.14 (d, 1H, J = 8.4 Hz, Ph), 7.13 (d, 1H, J = 7.2 Hz, Ph), 6.88 (m, 3H, Ph), 6.84 (m, 2H, Ph), 6.81 (m, 1H, Ph), 5.40 (t, 1H, J = 6.0 Hz, NH), 4.60, 4.43, 4.39, 4.38, 4.34, 4.26 (each d, each 1H, J = 11.4 Hz, CH_2Ph), 3.81 (s, 3H, OCH_3), 3.77, 3.75 (each s, each 3H, 2× OCH_3), 3.61 (m, 1H, OCH_2), 3.35 (m, 1H, OCH_2), 3.04 (m, 2H, CH_2NH), 2.86 (s, 6H, $N(CH_3)_2$), 2.11, 2.09, 2.01, 2.01 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.5, 170.0, 169.9, 169.5 (4× *C*OCH_3), 159.3, 159.2, 159.1 (C), 148.1, 134.8 (C), 130.3 (CH), 130.2 (C), 129.8 (CH), 129.7 (C), 129.6, 129.5, 128.2, 123.2, 118.9, 115.2, 113.8, 113.7, 113.6 (CH), 72.9, 71.6, 71.5 (3× *C*H_2Ph), 65.7 (OCH_2), 55.2, 55.2, 55.1 (3× OCH_3), 45.4 (NCH_3), 42.9 (CH_2NH), 20.8, 20.7, 20.6, 20.5 (4× CO*C*H_3).

3.1.1.34. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-β-D-galactofuranoside (46). A solution of **45** (150 mg, 0.13 mmol) and thiophenol (0.05 mL, 0.46 mmol) in dry CH_2Cl_2 (10 mL) was cooled to −78 °C under argon. $SnCl_4$ (0.05 mL, 0.40 mmol) was added and the mixture was stirred for 1 h at −78 °C and further overnight at −20 °C. A saturated $NaHCO_3$ solution (5 mL) was added and the reaction mixture was further diluted with $CHCl_3$ (30 mL). The organic layer was washed with water (2× 10 mL), dried over Na_2SO_4, and concentrated to an oil. Chromatography ($CHCl_3$/MeOH, 95:5) yielded **46** (85 mg, 83%) as an oil. FABMS (NBA): *m/z* 787 $[M+H]^+$. Anal. Calcd for $C_{34}H_{46}N_2O_{17}S{\cdot}1.0\ H_2O$: C, 50.74; H, 6.01; N, 3.48. Found: C, 50.77; H, 6.00; N, 3.44. ^1H NMR (300 MHz, $CDCl_3$, D_2O exchanged): non-sugar signals, δ 8.54 (d, 1H, J = 8.6 Hz, Ph), 8.26 (d, 1H, J = 8.6 Hz, Ph), 8.54 (dd, 1H, J = 1.2, 7.4 Hz, Ph), 7.54 (m, 2H, Ph), 7.19 (d, 1H, J = 7.4 Hz, Ph), 5.65 (br s, 1H, NH), 3.68 (m, 1H, OCH_2), 3.40 (m, 1H, OCH_2), 3.08 (dd, 2H, J = 4.6, 5.4 Hz, CH_2NH), 2.89 (s, 6H, $N(CH_3)_2$), 2.14, 2.12, 2.07, 2.06 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.7, 170.3, 170.1, 170.0 (4× *C*OCH_3), 151.9, 134.7 (C), 130.5 (CH), 129.9, 129.5 (C), 129.4, 128.4, 123.2, 118.8, 115.2 (CH), 65.8 (OCH_2), 43.00 (CH_2NH), 31.8, 29.4 ($N(CH_3)_2$), 20.8, 20.76, 20.72, 20.6 (4× CO*C*H_3).

3.1.1.35. 5-Azidonaphthalene-1-sulfonamidoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-2,3,5-tri-*O*-*p*-methoxybenzyl-β-D-galactofuranoside (47). Compound **44** (2.2 g, 2.41 mmol) was reacted with *N*-methylimidazole (0.38 mL, 4.82 mmol) and 1-azido-5-naphthalenesulfonyl chloride (967 mg, 0.40 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C for 4 h as described for **41**. Purification by column chromatography (cyclohexane/EtOAc, 1:1) gave disaccharide **47** (1.64 g, 68%) as an oil. ESI-MS: *m/z* 807.40 $[M+Na]^+$. Anal. Calcd for $C_{56}H_{64}N_4O_{20}S$: C, 58.73; H, 5.63; N, 4.89. Found C, 58.76; H, 5.69; N, 4.87. ^1H NMR (300 MHz, $CDCl_3$): non-sugar signals, δ 8.40 (d, 1H, J = 8.7 Hz, Ph), 8.37 (d, 1H, J = 8.3 Hz, Ph), 8.27 (dd, 1H, J = 1.0, 7.2 Hz, Ph), 7.53 (m, 2H, Ph), 7.27 (m, 1H, Ph), 7.17 (m, 6H, Ph), 6.84 (m, 6H, Ph), 5.48 (t, 1H, J = 5.8 Hz, NH), 4.60, 4.42, 4.37, 4.31, 4.26, 4.22 (each d, each 1H, J = 11.4 Hz, CH_2Ph), 3.82, 3.78, 3.76 (each s, each 3H, 3× OCH_3), 3.56 (m, 1H, OCH_2), 3.33 (m, 1H, OCH_2), 3.08 (m, 2H, CH_2NH), 2.12, 2.09, 2.02, 2.01 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.6, 170.1, 170.00, 169.5 (*C*OCH_3), 159.3, 159.3, 159.2 (C), 137.5, 134.8 (C), 130.3 (CH), 130.2 (C), 129.8 (CH), 129.6 (C), 129.5, 129.4 (CH), 129.3, 129.1 (C), 128.4, 128.1 (CH), 127.1 (C), 124.3, 121.0, 114.8, 113.8, 113.7, 113.6 (CH), 72.9, 71.7, 71.5 (3× *C*H_2Ph), 65.6 (OCH_2), 55.2, 55.2, 55.1 (3× OCH_3), 43.0 (CH_2NH), 20.8, 20.7, 20.6, 20.5 (4× CO*C*H_3).

3.1.1.36. 5-Azidonaphthalene-1-sulfonamidoethyl 6-*O*-(2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranosyl)-β-D-galactofuranoside (48). Disaccharide **47** (1.30 g, 1.14 mmol) was dissolved in 110 mL of CH_2Cl_2/H_2O (10:1) and DDQ (1.30 g, 5.7 mmol) was added at room temperature. The reaction mixture was stirred for 4 h, dried over Na_2SO_4, and concentrated to syrup. Column chromatography ($CHCl_3$/MeOH, 95:5) afforded **48** (720 mg, 81%) as oil. FABMS (NBA): *m/z* 639.14 $[M+Na]^+$. Anal. Calcd for $C_{32}H_{40}N_4O_{17}S{\cdot}1.0\ H_2O$: C, 48.42; H, 5.21; N, 7.06. Found: C, 48.46; H, 5.27; N, 7.01. ^1H NMR (600 MHz, $CDCl_3$): non-sugar signals δ 8.41 (d, 1H, J = 8.6 Hz, Ph), 8.40 (d, 1H, J = 8.3 Hz, Ph), 8.28 (dd, 1H, J = 1.0, 7.3 Hz, Ph), 7.65 (dd, 1H, J = 7.6, 8.6 Hz, Ph), 7.55 (dd, 1H, J = 7.3, 8.3 Hz, Ph), 7.37 (d, 1H, J = 7.6 Hz, Ph), 5.87 (t, 1H, J = 6.0 Hz, NH), 4.23 (br s, 1H, OH), 3.68 (m, 1H, OCH_2), 3.61 (br s, 1H, OH), 3.41 (m, 1H, OCH_2), 3.21 (d, 1H, J = 8.3 Hz, OH), 3.09 (dd, 2H, J = 5.3, 10.4 Hz, CH_2NH), 2.14, 2.12, 2.07, 2.06 (each s, each 3H, 4× $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.8, 170.4, 170.1, 17.1 (*C*OCH_3), 137.6, 134.7 (C), 130.2 (CH), 129.1 (C), 128.5, 128.2 (CH), 127.1 (C), 124.3, 122.0, 114.9 (CH), 65.8 (OCH_2), 42.8 (CH_2NH), 20.8, 20.77, 20.72, 20.6 (4× CO*C*H_3).

3.1.1.37. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside (1). Compound **20** (540 mg, 0.69 mmol) was dissolved in dry THF (20 mL) and tetraethylammonium fluoride (3.9 mg, 2.07 mmol) was added. The reaction mixture was stirred overnight at rt and concentrated to oil. Column chromatography ($CHCl_3$/MeOH, 5:1) gave compound **1** as an oil that was dissolved in deionized water (5 mL), frozen, and lyophilized to yield a light yellow, hygroscopic solid (372 mg, 97%). Spectral and analytical data have been reported.[13]

3.1.1.38. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (2). Compound **40** (125 mg, 0.13 mmol) was reacted with thiophenol (0.05 mL, 0.46 mmol) and

$SnCl_4$ (0.05 mL, 0.40 mmol) in dry CH_2Cl_2 (10 mL) as described for **46**. Column chromatography ($CHCl_3$/MeOH 3:1) gave **2** as a light sensitive oil. An aqueous solution (5 mL) of the oil was passed through a small column packed with Bio-Beads^{TM} SM-4 (20–50 mesh), and the solution was frozen and lyophilized to afford **2** as a hygroscopic solid (27 mg, 33%). FABMS (NBA): *m/z* 619 $[M+H]^+$. Anal. Calcd for $C_{26}H_{38}N_2O_{13}S{\cdot}1.0\ H_2O$: C, 49.05; H, 6.33; N, 4.40. Found C, 49.00; H, 6.36; N, 4.38. ^{1}H NMR (600 MHz, CD_3OD): non-sugar signals, δ 8.56 (dt, 1H, J = 1.2, 8.4 Hz, Ph), 8.34 (dt, 1H, J = 0.6, 9.0 Hz, Ph), 8.20 (dd, 1H, J = 1.2, 7.2 Hz, Ph), 7.59 (dd, 1H, J = 8.4, 9.0 Hz, Ph), 7.58 (dd, 1H, J = 8.4, 9.0 Hz, Ph), 7.28 (dd, 1H, J = 0.6, 7.2 Hz, Ph), 3.55 (m, 1H, OCH_2), 3.31 (m, 1H, OCH_2), 3.05 (dd, 2H, J = 5.4, 7.2 Hz, CH_2NH), 2.88 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (75 MHz, CD_3OD): non-sugar signals, δ 153.2, 137.1 (2× C), 131.2 (CH), 131.2, 130.9 (2× C), 130.0, 129.1, 124.3, 120.6, 116.5 (5× CH), 67.6 (OCH_2), 45.8 ($N(CH_3)_2$), 43.9 (CH_2NH).

3.1.1.39. 5-*N*,*N*-Dimethylaminonaphthalene-1-sulfonamidoethyl 6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (3). Disaccharide **46** (80 mg, 0.10 mmol) was deacetylated by 7 N NH_3/MeOH (5 mL) as described for **40**. Column chromatography ($CHCl_3$/MeOH, 3:1) followed by a short column of Bio-Beads^{TM} SM-4 (20–50 mesh), freezing, and lyophilization afforded **3** as yellow fluffy solid (44 mg, 70%). FABMS (NBA): *m/z* 619 $[M+H]^+$. Anal. Calcd for $C_{26}H_{38}N_2O_{13}S{\cdot}1.0\ H_2O$: C, 49.05; H, 6.33; N, 4.40. Found C, 48.99; H, 6.32; N, 4.37. ^{1}H NMR (600 MHz, CD_3OD): non-sugar signals, δ 8.56 (1H, dt, J = 1.2, 8.4 Hz, Ph), 8.34 (1H, dt, J = 1.2, 8.4 Hz, Ph), 8.20 (1H, dd, J = 1.2, 7.2 Hz, Ph), 7.59 (1H, dd, J = 7.2, 8.4 Hz, Ph), 7.58 (1H, dd, J = 7.2, 8.4 Hz, Ph), 7.28 (1H, dd, J = 1.2, 8.4 Hz, Ph), 3.59 (1H, m, OCH_2), 3.31 (1H, m, OCH_2), 3.06 (2H, t, J = 5.4 Hz, CH_2NH) 2.88 (6H, s, $N(CH_3)_2$). ^{13}C NMR (75 MHz, CD_3OD): non-sugar signals, δ 153.2, 137.1 (2× C), 131.2 (CH), 131.2, 130.9 (2× C), 130.0, 129.1, 124.3, 120.6, 116.5 (5× CH), 67.5 (OCH_2), 45.8 ($N(CH_3)_2$), 43.9 (CH_2NH).

3.1.1.40. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside (4). Compound **25** (300 mg, 0.38 mmol) was dissolved in dry THF (20 mL) and $Et_4N^+F^-$ (171 mg, 1.15 mmol) was added. The reaction mixture was stirred overnight and concentrated to oil. Column chromatography ($CHCl_3$/MeOH, 5:1) gave compound **4** as an oil that was dissolved in deionized water (5 mL), frozen, and lyophilized to get a light sensitive, hygroscopic solid (128 mg, 60%). Spectral and analytical data have been reported.[14]

3.1.1.41. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (5). A solution of compound **42** (610 mg, 0.78 mmol) in dry MeOH (15 mL) was cooled to 0 °C under argon and treated with 7 N NH_3/MeOH. The reaction mixture was stirred overnight at room temperature and concentrated to dryness. An aqueous solution (5 mL) was passed through a small column of Bio-Beads™ SM-4 (25 g, 20–50 mesh) and product **5** was eluted with MeOH/H_2O (0/100%). The aqueous solution that remained after brief evaporation was frozen and lyophilized to yield a light sensitive solid (375 mg, 78%). ESI-MS: *m/z* 639.17 $[M+Na]^+$. Anal. Calcd for $C_{24}H_{32}N_4O_{13}S{\cdot}1.0\ H_2O$: C, 45.42; H, 5.40; N, 8.53. Found C, 45.49; H, 5.39; N, 8.61. ^{1}H NMR (600 MHz, CD_3OD): non-sugar signals δ 8.49 (dt, 1H, J = 0.8, 0.9, 8.7 Hz, Ph), 8.40 (dt, 1H, J = 0.9, 1.2, 8.5 Hz, Ph), 8.26 (dd, 1H, J = 1.2, 7.3 Hz, Ph), 7.71 (dd, 1H, J = 7.6, 8.7 Hz, Ph), 7.60 (dd, 1H, J = 7.4, 8.5 Hz, Ph), 7.48 (dd, 1H, J = 0.8, 7.6 Hz, Ph), 3.51 (m, 1H, OCH_2), 3.28 (m, 1H, OCH_2), 3.07 (t, 2H, J = 5.6 Hz, CH_2NH). ^{13}C NMR (75 MHz, CD_3OD): 138.8, 137.1 (C), 130.9 (CH), 130.4 (C), 129.1 (CH), 128.4 (C), 125.5, 122.6, 116.1 (CH), 67.5 (OCH_2), 43.9 (CH_2NH).

3.1.1.42. 5-Azidonaphthalene-1-sulfonamidoethyl 6-*O*-(β-D-galactofuranosyl)-β-D-galactofuranoside (6). Disaccharide **48** (700 mg, 0.89 mmol) was deacetylated in 7 N NH_3/MeOH (20 mL) as described for the preparation of **40**. An aqueous solution (5 mL) of crude disaccharide was passed through a small column of Bio-Beads™ SM-4 (25 g, 20–50 mesh) and eluted with MeOH/H_2O (0–100%). The aqueous solution that remained after brief evaporation was frozen and lyophilized to a solid (439 mg, 80%). ESI-MS: *m/z* 619 $[M+H]^+$. Anal. Calcd for $C_{24}H_{32}N_4O_{13}S{\cdot}1.0\ H_2O$: C, 45.42; H, 5.40; N, 8.53. Found C, 45.49; H, 5.41; N, 8.57. ^{1}H NMR (600 MHz, CD_3OD): non-sugar signals, δ 8.50 (d, 1H, J = 8.5 Hz, Ph), 8.41 (d, 1H, J = 8.5 Hz, Ph), 8.27 (dd, 1H, J = 1.2, 7.4 Hz, Ph), 7.72 (dd, 1H, J = 7.6, 8.7 Hz, Ph), 7.62 (dd, 1H, J = 7.4, 8.5 Hz, Ph), 7.50 (d, 1H, J = 7.6 Hz, Ph), 3.56 (m, 1H, OCH_2), 3.29 (m, 1H, OCH_2), 3.08 (t, 2H, J = 5.5 Hz, CH_2NH). ^{13}C NMR (75 MHz, CD_3OD): non-sugar signals, δ 138.8, 137.2 (C), 130.9 (CH), 130.5 (C), 129.1 (CH), 128.4 (C), 125.5, 122.6, 116.1 (CH), 67.4 (OCH_2), 43.9 (CH_2NH).

3.2. Biological activity

3.2.1. Arabinosyltransferase assay. Compounds **1** at a range of concentrations from 0.25 to 2.0 mM and **2** from 0.5 to 8.0 mM stored as 100 mM ethanol stocks and DP[^{14}C]A (20,000 cpm, 9 mM, 10 μL [stored in chloroform/methanol, 2:1]) 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 dried constituents of the assay were then resuspended in 8 μl of a 1% aqueous solution of Igepal. The remaining constituents of the arabinosyltransferase assay containing 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM ATP, and *M. smegmatis* membranes (250 μg) were added to 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_20 (3 mL). The resulting organic phase was recovered following centrifugation at 3500*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). The incorporation of [^{14}C]Ara*f* 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 aluminum backed Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). Autoradiograms were obtained by exposing TLCs to X-ray film (Kodak X-Omat) for 3 days.

3.2.2. Galactosyltransferase assay. All four disaccharides at a range of concentrations from 0.5 to 6.0 mM were incubated with 0.5 μCi of UDP-[^{14}C]Gal*p* (Amersham, 257 mCi/mmol) in a buffer containing 50 mM MOPS, pH 7.9, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 62.5 μM ATP, and 50 μL of a membrane preparation from *M. smegmatis* (0.5 mg of protein) in a total volume of 160 μL for 1 h at 37 °C. A chloroform/methanol (1:1, 1.07 mL) solution was then added to the incubation tubes and the entire contents centrifuged at 14,000 rpm. The supernatant was recovered, dried under a stream of argon, re-suspended in ethanol:water (1:1, 1 mL), and loaded onto a 1 mL Whatman strong anion exchange (SAX) cartridge. The cartridge was then washed with ethanol (3 mL). The eluants were combined, dried, and the resulting products partitioned between the two phases arising from *n*-butanol (3 mL) and water (3 mL). The organic phase was recovered and back washed with water saturated with *n*-butanol twice (3 mL each). The *n*-butanol fraction was dried, re-suspended in 200 μL of *n*-butanol-saturated with water. Fifty microliters of this solution was subjected to scintillation counting, and another 50 μL was applied to an analytical thin-layer chromatogram developed in chloroform/methanol/ammonium hydroxide/water (65:25:0.5:3.6) and subjected to autoradiography. 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 acceptors).

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References and notes

1. (a) Rattan, A.; Kalia, A.; Ahmad, N. *Emerging Infect. Dis.* **1998**, *4*, 195–209; (b) Butler, D. *Nature* **2000**, *406*, 670–672; (c) Raviglione, M. C.; Snider, D. E.; Kochi, A. *JAMA* **1995**, *273*, 220–226; (d) NIAID, Web Site, 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.
2. (a) Van Scoy, R. E.; Wilkowske, C. J. *Mayo Clin. Proc.* **1999**, *74*, 1038–1048; (b) Ravilione, M. C. *Scot. Med. J.* **2000**, *45*, 52–55; (c) Long, R. *CMAJ* **2000**, *163*, 425–428.
3. (a) Walsh, C. *Nature* **2000**, *406*, 775–781; (b) Smith, M. B.; Boyaromatics, M. C.; Veasey, S.; Woods, G. L. *Aromaticch. Pathol. Lab. Med.* **2000**, *124*, 1267–1274; (c) Pozniak, A. *Int. J. Tuberc. Lung Dis.* **2000**, *4*, 993–994.
4. De Jong, B. C.; Israelski, D. M.; Corbett, E. L.; Small, P. M. *Annu. Rev. Med.* **2004**, *55*, 283–301.
5. Sibille, Y.; Reynolds, H. *Am. Rev. Respir. Dis.* **1990**, *141*, 47–50.
6. (a) Mauch, H. *Zentralblzhyg Umweltmed.* **1993**, *194*, 152–161; (b) Bass, J. B.; Farer, I. S.; Hopewell, P. C.; Obrien, R.; Jacobs, R. F.; Ruben, F.; Snider, D. E.; Thornton, G. *Am. J. Respir. Crit. Care Med.* **1994**, *149*, 1359–1371.
7. (a) Blanchard, J. S. *Annu. Rev. Biochem.* **1996**, *65*, 215–239; (b) Lowary, T. L. Mycobacterial Cell Wall Components. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; pp 2005–2080.
8. (a) Abou-Zeid, C.; Voiland, A.; Michel, G.; Cocito, C. *Eur. J. Biochem.* **1982**, *128*, 363–370; (b) Daffe, M.; Brennan, P. J.; McNeil, M. *J. Biol. Chem.* **1990**, *265*, 6734–6743; (c) Brennen, P. J.; Nikaido, H. *Annu. Rev. Biochem.* **1995**, *64*, 29–63; (d) Besra, G. S.; Khoo, K.-H.; McNeil, M. R.; Dell, A.; Morris, H. R.; Brennan, P. *Biochemistry* **1995**, *34*, 4257–4266; (e) Lee, R. E.; Brennan, P. J.; Besra, G. S. *Curr. Top. Microbiol. Immunol.* **1996**, *215*, 1–27; (f) Mikusova, K.; Yagi, T.; Stern, R.; McNeil, M. R.; Besra, G. S.; Crick, D. C.; Brennan, P. J. *J. Biol. Chem.* **2000**, *275*, 33890.
9. Mikusova, K.; Beláňová, M.; Korduláková, J.; Honda, K.; McNeil, M. R.; Mahapatra, S.; Crick, D. C.; Brennan, P. J. J. *Bacteriology* **2006**, *188*, 6592–6598.
10. (a) 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; (b) Reynolds, R. C.; Bansal, N.; Rose, J.; Friedrich, J.; Suiling, W. J.; Maddry, J. A. *Carbohydr. Res.* **1999**, *317*, 164–179; (c) Pathak, A. K.; Pathak, V.; Maddry, J. A.; Suling, W. J.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Bioorg. Med. Chem.* **2001**, *9*, 3145–3151; (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; (e) Pathak, A. K.; Pathak, V.; Suling, W. J.; Gurcha, S. S.; Morehouse, C. B.; Besra, G. S.; Maddry, J. A.; Reynolds, R. C. *Bioorg. Med. Chem.* **2002**, *10*, 923–928; (f) Pathak, A. K.; Pathak, V.; Kulshrestha, M.; Kinnaird, D.; Suling, W. J.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Tetrahedron* **2003**, *59*, 10239–10248; (g) Kremer, L.; Dover, L. G.; Gurcha, S. S.; Pathak, A. K.; Reynolds, R. C.; Besra, G. S. *Carbohydr. Bioeng.* **2002**, 178–185.
11. (a) Bayley, H.; Knowles, J. R. *Methods Enzymol.* **1977**, *46*, 69–114; (b) Hazum, E. *Methods Enzymol.* **1983**, *103*, 58–

71; (c) Eberle, A. N.; deGraan, P. N. E. *Methods Enzymol.* **1985**, *109*, 129–156.
12. Cory, P. P.; Becker, R. R.; Rosenbluth, R.; Isenberg, I. *J. Am. Chem. Soc.* **1968**, 1643–1647.
13. (a) Muramoto, K.; Kamiya, H. *Agric. Biol. Chem.* **1984**, *48*, 2695–2699; (b) Muramoto, K.; Kamiya, H. *Agric. Biol. Chem.* **1988**, *52*, 547–554.
14. Pathak, A. K.; Pathak, V.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **2001**, *42*, 979–982.
15. Pathak, A. K.; Pathak, V.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2749–2752.
16. Pathak, A. K.; Pathak, V.; Riordan, J. M.; Gurcha, S. S.; Besra, G. S.; Reynolds, R. C. *Carbohydr. Res.* **2004**, *339*, 683–691.
17. (a) Guthrie, R. D.; Smith, S. C. *Chem. Ind. (London)* **1968**, 547–548; (b) Kam, B. L.; Barascut, J.-L.; Imbach, J.-L. *Carbohydr. Res.* **1979**, *69*, 135–142.
18. Pathak, A. K.; El-Kattan, Y. A.; Bansal, N.; Maddry, J. A.; Reynolds, R. C. *Tetrahedron Lett.* **1998**, *39*, 1497–1500.
19. Pathak, A. K.; Pathak, V.; Khare, N. K.; Maddry, J. A.; Reynolds, R. C. *Carbohydr. Lett.* **2001**, *4*, 117–122.
20. D'Souza, F. W.; Cheshev, P. E.; Ayers, J. D.; Lowary, T. L. *J. Org. Chem.* **1998**, *63*, 9037–9044.
21. Chittenden, G. J. F. *Carbohydr. Res.* **1972**, *25*, 35–41.
22. Greene, T. W.; Wuts, P. G. M. In *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley, 1999; pp 86–91.
23. Mizutani, K.; Kasai, R.; Nakamura, M.; Tanaka, O.; Matsuura, H. *Carbohydr. Res.* **1989**, *185*, 27–38.
24. Cyr, N.; Perlin, A. S. *Can. J. Chem.* **1979**, *57*, 2504–2511.
25. Lee, R. E.; Brennan, P. J.; Besra, G. S. *Glycobiology* **1997**, *7*, 1121–1128.
26. Rose, N. L.; Completo, G. C.; Lin, S.-J.; McNeil, M.; Palcic, M. M.; Lowary, T. L. *J. Am. Chem. Soc.* **2006**, *128*, 6721–6729.