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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 Araf(α 1 $\rightarrow$ 5)Araf, Gal(β 1 $\rightarrow$ 5)Gal and Gal(β 1 $\rightarrow$ 6)Gal 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 towards glycosyltransferases is also presented.

Keywords

arabinofuranose; galactofuranose; fluorescent probe; photoaffinity probe; glycosyltransferases; arabinosyltransferases; galactosyltransferases; tuberculosis

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

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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 drugable 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 which is attached the linear D-galactan composed of alternating 5- and 6-linked β -D-Galf 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 $\rightarrow 5$ -D-Galf-($\beta 1 \rightarrow 6$)-D-Galf-($\beta 1 \rightarrow 5$)-D-Galf-($\beta 1 \rightarrow 4$)-L-Rhap-($\alpha 1 \rightarrow 3$)-D-GlcpNAc. 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 (Manp), galactofuranose (Galf) and arabinofuranose (Araf) in a variety of defined glycosidic linkages. The two major oligosaccharide portions are AG and lipoarabinomannan (LAM). AG is composed of alternate ($\beta 1 \rightarrow 5$) and ($\beta 1 \rightarrow 6$) linked Galf units, linear ($\alpha 1 \rightarrow 5$) linked Araf and branched arabinan hexasaccharide at the terminus that consist of ($\alpha 1 \rightarrow 5$), ($\alpha 1 \rightarrow 3$), ($\beta 1 \rightarrow 2$) linked Araf units (Figure 1). LAM is Manp capped arabinan. The AG complex is critical for the survival of *M. 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 Figure 2). The galactan portion is prepared through the actions of a bifunctional galactosyltransferase (GalT) enzyme preparing both the ($\beta 1 \rightarrow 5$) and ($\beta 1 \rightarrow 6$) linkages along with another recently characterized enzyme (Rv3782), and both enzymes utilize the donor uridinediphosphate-galactofuranose (UDP-Galf) (represented in Figure 3).^{8,9} UDP-Galf is produced by the conversion of UDP-galactopyranose (UDP-Galp) by the enzyme UDP-Galp 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 a 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 generally 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-sulfonamido¹⁴ (dansyl) function (Chart 1). These substrates were designed for the development of non-radiolabelled second generation competitive based acceptor assays that can potentially eliminate the use of radiolabelled donors (DPA and UDP-Galp). Furthermore, this paper describes the preparation of related disaccharides (**4** – **6**) bearing 1-azido-5-naphthalene sulfonamido¹³ 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. Result and Discussion

2.1 Strategy

We have previously described syntheses of Ara β and Gal β 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 β disaccharides¹⁴ and related photoaffinity probes,¹⁵ and a Man α photoaffinity probe¹⁶ 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 β disaccharide preparation to introduce an ethylamino functionality for attachment of naphthylsulfonamido group at the anomeric center (Figure 4). Starting from the readily synthesized Ara β 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 yield. Unfortunately, deacetylation of **8** using several different reagents proved ineffective, and only a poor yield (48%) of deacetylated glycoside was achieved using K₂CO₃ 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 $\rightarrow$ 5-linked disaccharide **9**. The de-phthaloylation 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 $\rightarrow$ 5 linked disaccharide. This approach was also utilized to prepare the 1-*O*-ethylazido glycosides in the Gal β disaccharide probes. The fluorescent and photoaffinity disaccharides Ara β (α 1 $\rightarrow$ 5)Ara β , Gal β (β 1 $\rightarrow$ 5)Gal β and Gal β (β 1 $\rightarrow$ 6)Gal β 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 SnCl₄ 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 NaN₃ in dry DMF for 6 hours followed by deacetylation with 7N NH₃/MeOH to give **12** in an overall yield of 70% (2 steps) after purification. The 5-position was selectively blocked using 1.0 eq. 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 7N NH₃/MeOH to obtain the acceptor **15** in high yield. The trichloroacetimidate donor **16** was synthesized as reported.¹⁹

2.2.1 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 hours in the presence of the Lewis acid promoter BF₃•Et₂O (1.0 eq., dissolved in 2 mL of dry CH₂Cl₂). Additions were done at 0 °C, and the reaction was carried out under an inert atmosphere in dry CH₂Cl₂ at room temperature over powdered 4Å molecular sieves. Column chromatography on Silica gel G afforded the pure disaccharide **17** in 66% yield. The azido group was reduced by heating with Ph₃P in a benzene/H₂O mixture followed by rapid purification *via* flash chromatography to afford compound **18**.

2.2.2 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 high yield. Lastly, compound **20** obtained from **19**, was de-protected to give the final fluorescent target **1**.

2.3 Synthesis of photo affinity probe for AraT's (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 eq. 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, were carried out at –20 °C under an argon atmosphere in dry CH₂Cl₂ 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 ¹HNMR spectrum.¹⁵ The azido group was reduced by reaction with ammonium formate (H₂CO₂NH₄) 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

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 Galf(β 1 $\rightarrow$ 5)Galf(**2**) and a Galf(β 1 $\rightarrow$ 5)Galf(**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 NaN₃ in DMF at 70 °C gave the glycoside **28** possessing the ethylazido functionality. Compound **28** was deacetylated using 7N NH₃/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-camphorsulphonic 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, de-tritylation with 10% CF₃COOH 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 Galf(β 1 $\rightarrow$ 5)Galf disaccharide: The synthesis of β 1 $\rightarrow$ 5 linked Galf 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 H₂CO₂NH₄ 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 β 1 $\rightarrow$ 5 linked Galf probes.

2.4.2.2. Synthesis of Galf(β 1 $\rightarrow$ 6)Galf disaccharide: The synthesis of the β 1 $\rightarrow$ 6 linked Galf disaccharide was carried out by coupling glycoside **36** and the thiocresyl 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 $\text{H}_2\text{CO}_2\text{NH}_4$ 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 photo affinity probes for GT

2.5.1. Galf(β 1 $\rightarrow$ 5)Galf fluorescent disaccharide (2) and photo affinity 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 de-acetylated 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 conditions²² 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 debenzoylation under conditions used to prepare **2** gave the target **5** in 78 % yield.

2.5.2. Galf(β 1 $\rightarrow$ 6)Galf fluorescent disaccharide (3) and photoaffinity probe (6)

—Synthesis of fluorescently labeled β 1 $\rightarrow$ 6 linked Galf disaccharide **3** and photo affinity probe **6** were carried out in a similar fashion to the β 1 $\rightarrow$ 5 linked Galf 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 Araf 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 Araf disaccharides the H-1 anomeric protons of the reducing sugar (4.58 – 4.83 ppm) are generally seen at lower ppm as compare to non-reducing sugar H-1' (4.87 – 5.38 ppm) with coupling constant $^3J_{1,2}$ and $^3J_{1',2'} = 2.0$ 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 compare to C-1' in the range 110 – 105 ppm. The β -stereochemistry, *trans*-glycosylation, of all of the D-Galf 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 Galf 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 $^3J_{1,2} = 2.0$ Hz. As with the Araf disaccharides, the anomeric proton of the reducing sugar in Galf disaccharides is seen at

higher field as compared to a non-reducing sugar. In ^{13}C NMR spectra of Galf glycosides the C-1 signal appeared between 108.6 – 105.4 ppm. However, in Galf disaccharides the C-1 proton is seen between 105.8 – 109.9 ppm and C-1' at higher field in a range between 105.2 – 109.5 ppm. Detailed NMR data (^1H and ^{13}C NMR) on all disaccharides (sugar portion) are presented in Table 1 – 4 and the values confirmed the proposed configuration at the anomeric centers and the structures of synthesized disaccharides.

2.8. 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 *M. smegmatis* was used to evaluate their acceptor activity.^{10c,d,24}

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

2.8.2. Galactosyltransferases activity—All four Galf 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 β incorporation from UDP-[^{14}C]Gal β , following endogenous conversion to UDP-[^{14}C]Gal β and transferase activity for all Galf probes. Different concentrations of probes **2** and **3** in the range of 0 to 4 mM were used for galactosyltransferase activity. In the case of probes **5** and **6**, a concentration range of 0 to 6 mM was utilized. TLC/autoradiography (Figure 6 and 8) demonstrated acceptor activity and the likely enzymatic conversion of all Galf 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**. Galf disaccharides **3**, **4** and **6** gave rise to a second, slower migrating band (Figure 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 (Figure 7 and 9) revealed that **2** and **3** possessed K_m values 7.33 mM and 2.38 mM, respectively whereas **5** and **6** possessed K_m values 2.77 mM and 1.98 mM, respectively. The acceptor data clearly suggested that disaccharide probes **2** and **3** can be used for the development of competitive-based assays for inhibitor discovery, and that disaccharides **5** and **6** may be useful for probing the bi-functional galactosyltransferase.

2.9. Conclusion

In summary, we reported a detailed account of the syntheses of Ara β (a1 $\rightarrow$ 5) Ara β , Gal β (b1 $\rightarrow$ 5) Gal β and Gal β (b1 $\rightarrow$ 6) Gal β 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 (¹⁴C-DPA) for AraT's and UDP-[¹⁴C]Galp (used for *in situ* preparation of UDP-[¹⁴C]Galp) for GalT. Recently, we have reported the use of Galp (β1? 5) Galp and Galp (β1? 6) Galp disaccharides as potential photoaffinity probes to identify the galactosyltransferase (*glfT*) in MTB.[§] Very recently the isolation and purification of *glfT* 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 SECTION

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 (60F₂₅₄) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spray with (NH₄)₂SO₄ solution (150 g ammonium sulfate, 30 mL H₂SO₄, 750 mL H₂O). All solvents used for work-up and chromatography were reagent grade. Flash column chromatography was carried out on silica gel 60 (230–400 Mesh). ¹H and ¹³C NMR spectra were recorded at 300 MHz and 75 MHz respectively. Specific ¹H NMR 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 ¹H NMR as per journal requirements.

3.1.2. Chloroethyl 2,3,5-tri-*O*-acetyl-α-D-arabinofuranoside (11)—To a dry CH₃CN (800 mL) solution of 2,3,5,6-tetra-*O*-acetyl-α-D-arabinofuranoside **10** (26.0 g, 81.69 mmol) was added SnCl₄ 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 gm) was added, the suspension was cooled in an ice-water bath, and a saturated aqueous NaHCO₃ 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 NaHCO₃ (2×100 mL) followed by water (100 mL) and brine (100 mL). The organic layer was dried over Na₂SO₄ and concentrated to an oil. Column chromatography (CHCl₃ 100%) afforded pure α-isomer **11** (22.1 g, 85%) as an oil. FABMS: *m/z* 525 [M+H]⁺. Anal. calcd for C₁₃H₁₉ClO₈: 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, OCH₂), 3.87 (m, 3H, OCH₂, CH₂Cl).

3.1.3. Azidoethyl α-D-arabinofuranoside (12)—To a dry DMF (500 mL) solution of compound **11** (31.0 g, 91.52 mmol) was added NaN₃ (8.9 g, 137.28 mmol). The reaction

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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. 7N NH₃/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 CHCl₃ – MeOH (95:5) as the eluting solvent to yield compound **12** (14.4 g, 70%). FABMS: *m/z* 220 [M+H]⁺. Anal. calcd for C₇H₁₃N₃O₅ • 1.0 H₂O: 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 (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, OCH₂), 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, OCH₂), 3.46 (dd, 1H, *J*_{4,5b} 5.7 Hz, *J*_{5a,5b} 11.9 Hz, H-5b), 3.44 (m, 2H, CH₂N₃).

3.1.4. 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 in 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 CHCl₃ (200 mL). The organic layer was washed with water (2×50 mL), dried over Na₂SO₄ and concentrated to oil. Column chromatography using CHCl₃ – MeOH (98:2) as the eluting solvent gave compound **13** (12.4 g, 58%). FABMS (LiCl): *m/z* 330 [M+Li]⁺. Anal. calcd for C₁₄H₁₇N₃O₆ • 1.0 H₂O: C, 49.25; H, 5.61; N, 12.31. Found C, 49.29; H, 5.58; N, 12.29. ¹H 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, OCH₂), 3.59 (m, 1H, OCH₂), 3.40 (m, 2H, CH₂N₃).

3.1.5. 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 was added TBDMSCl (21.5 g, 141 mmol) and imidazole (19.2 g, 282 mmol). The reaction mixture was heated at 60 °C for one days and concentrated. The residue was dissolved in CHCl₃ (40 mL) and washed with water (10 mL). Organic layer was dried over Na₂SO₄ 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 C₂₆H₄₅N₃O₆Si₂: C, 56.59; H, 8.23; N, 7.62. Found C, 56.58; H, 8.19; N, 7.70. ¹H NMR (CDCl₃): 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, OCH₂), 3.59 (m, 1H, OCH₂), 3.46 (m, 1H, CH₂N₃), 3.35 (m, 1H, CH₂N₃).

3.1.6. Azidoethyl 2,3-O-di-*tert*-butyldimethylsilyl-α-D-arabinofuranoside (15)

Method A: Compound **14** (17.0 g, 30.52 mmol) was dissolved in 7N NH₃/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 – H₂O (1:1, 17.5 mL) at 0 °C and the solution was stirred for 4 h. A saturated aqueous solution of NaHCO₃ (20 mL) was added and the mixture was diluted with CHCl₃ (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

3.1.7. Azidoethyl 5-O-(2,3,5-tri-O-benzoyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (17)—A dry CH₂Cl₂ (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 BF₃·Et₂O (0.34 mL, 2.69 mmol) was added dropwise and the reaction was stirred for another 2 h at –20 °C. The reaction mixture was poured into a cold, saturated solution of NaHCO₃ (20 mL). The organic layer was washed with water (2×25 mL), brine (20 mL) and dried over Na₂SO₄. 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

3.1.8. Aminoethyl 5-O-(2,3,5-tri-O-benzoyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (18)—Compound **17** (1.0 g, 1.16 mmol) was dissolved in 50 mL of benzene, Ph₃P (608 mg, 2.32 mmol) was added, and the reaction was heated to 50 °C. After 1 hr, water (2.8 mL) was added and heating was continued for 5 h. The reaction mixture was concentrated and dissolved in CHCl₃, washed with water (2×10 mL) and brine (10 mL). The organic layer was dried over Na₂SO₄, concentrated and chromatographed (CHCl₃ – MeOH, 95:5) to give **18** (845 mg, 87%) as a colorless oil. Spectral and analytical data have been reported.¹³

3.1.9. 5-N,N-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-O-(2,3,5-tri-O-benzoyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (19)—To a dry CH₂Cl₂ (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 was stirred at 0 °C for 3 h. The mixture was washed with water and brine, and the organic layer was dried over Na₂SO₄. Concentration 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

3.1.10. 5-N,N-Dimethylaminonaphthalene-1-sulfonamidoethyl 5-O-(a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (20)—Compound **19** (900 mg, 0.82 mmol) was debenzoylated with 7N NH₃/MeOH (30 mL) as described for the preparation of **15**. Purification by column chromatography (CHCl₃ – MeOH, 96:4) afforded **20** (567 mg, 88%) as a light yellow oil. FABMS (LiCl): *m/z* 793.5 [M+Li]⁺. Anal. calcd for C₃₆H₆₂N₂O₁₁SSi₂: C, 54.94; H, 7.95; N, 3.56. Found: C, 54.98; H, 7.92; N, 3.50. ¹H NMR (CDCl₃): 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, D₂O exchangeable), 3.50 (m, 2H, OCH₂), 3.25 (d, 1H, *J* 11.4 Hz, 3'-OH), 3.08 (m, 2H, CH₂NH), 2.89 (s, 2H, 2xNCH₃), 0.87, 0.86 (s, 9H each 6xCH₃), 0.08 (s, 3H, CH₃), 0.06 (s, 6H, CH₃), 0.05 (s, 3H, CH₃). ¹³C NMR (CDCl₃): 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 (OCH₂), 45.4 (NCH₃), 43.3 (CH₂NH), 25.7, 25.7 (CH₃), 17.83, 17.82 (C), –4.3, –4.61, –4.67, –4.8 (CH₃).

3.1.11. Azidoethyl 2,3,5-tri-O-tert-butylidimethylsilyl-a-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

3.1.12. Azidoethyl 5-O-(2,3,5-tri-O-acetyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (22)—Alcohol **15** (980 g, 2.05 mmol) and thioglycoside donor 1-Deoxy-1-thiocresyl-2,3,5-tri-O-acetyl-a-D-arabinofuranoside¹⁹ **7** (942 mg, 2.46 mmol) and activated, powdered 4Å molecular sieves (100 mg) in dry CH₂Cl₂ (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 Et₃N (5 mL), diluted with CH₂Cl₂ (60 mL) and filtered through a celite pad. The filtrate was washed with 10% Na₂S₂O₃ (20 mL), followed by washing with saturated aqueous NaHCO₃ (20 mL). The organic layer was dried over Na₂SO₄, 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 C₃₀H₅₅N₃O₁₂Si₂. ¹H NMR (CDCl₃): non-sugar signals, δ 3.88 (m, 1H, OCH₂), 3.57 (m, 1H, OCH₂), 3.44 (m, 1H, CH₂N₃), 3.34 (m, 1H, CH₂N₃), 2.10 (s, 6H, 2xCOCH₃), 2.09 (s, 3H, CH₃), 0.99 (s, 9H, 3xCH₃), 0.88 (s, 9H, 3xCH₃), 0.102 (9H, s, 3xCH₃), 0.099 (s, 6H, 2xCH₃), 0.081 (s, 3H, CH₃). ¹³C NMR (CDCl₃): non-sugar signals, δ 170.6, 170.1, 169.4 (COCH₃), 66.4 (OCH₂), 50.8 (CH₂N₃), 25.8, 25.7 (C), 20.75, 20.72, 20.7 (COCH₃), 17.83, 17.79 (CH₃), -4.3, -4.7, -4.8, -4.9 (CH₃).

3.1.13. Aminoethyl 5-O-(2,3,5-tri-O-acetyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-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. H₂CO₂NH₄ (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 was filtered through a short celite pad and concentrated to syrup. The concentrate was dissolved in CHCl₃ (20 mL), washed with deionized water (10 mL) and dried over Na₂SO₄. Evaporation of the organic layer afforded the crude disaccharide **23** (420 mg) that was used as such to prepare **24**. ¹H NMR (CDCl₃): major non-sugar signals in crude sample, δ 3.71 (m, 1H, OCH₂), 3.43 (m, 1H, OCH₂), 2.86 (m, 2H, CH₂NH), 2.10 (s, 6H, CH₃), 2.09 (s, 3H, CH₃), 0.90 (s, 12H, CH₃), 0.88 (s, 12H, CH₃), 0.10, 0.09, 0.08 (CH₃'s).

3.1.14. 5-azidonaphthalene-1-sulfonamidoethyl 5-O-(2,3,5-tri-O-acetyl-a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-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 CH₂Cl₂ (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

3.1.15. 5-azidonaphthalene-1-sulfonamidoethyl 5-O-(a-D-arabinofuranosyl)-2,3-O-di-tert-butylidimethylsilyl-a-D-arabinofuranoside (25)—Compound **24** (430 mg, 0.47 mmol) was deacetylated by 7N NH₃/MeOH (6 mL) as described for the preparation of **20**. Column chromatography (CHCl₃ – MeOH, 95:5) afforded pure **25** (322 mg, 87%) as a light sensitive, yellow oil. Spectral and analytical data have been reported.¹⁴

3.1.16. 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 SnCl₄ (6.0 mL, 51.28 mmol) and 1-chloroethanol (4.12 mL, 61.52 mmol) in dry CH₃CN (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 C₁₆H₂₃ClO₁₀: C, 46.78; H, 5.64. Found: C, 46.69; H, 5.60. ¹H NMR (300 MHz, CDCl₃) 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, OCH₂), 3.78 (m, 1H, OCH₂), 3.66 (m, 2H, CH₂Cl), 2.14, 2.12, 2.10, 2.07 (each s, each 3H, 4xCOCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.5, 170.00, 169.95, 169.6 (COCH₃), 105.5 (C-1), 81.3 (C-2), 80.1 (C-4), 76.3 (C-3), 70.0 (C-5), 67.7 (OCH₂), 62.4 (C-6), 42.6 (CH₂Cl), 20.8, 20.72, 20.68, 20.63 (4xCOCH₃).

3.1.17. 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 NaN₃ (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 CHCl₃ (200 mL), washed with water (2x40 mL), dried over Na₂SO₄ 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 C₁₆H₂₃N₃O₁₀: C, 46.04; H, 5.56; N, 10.07. Found: C, 46.06; H, 5.62; N, 9.99. ¹H NMR (300 MHz, CDCl₃): 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, OCH₂), 3.66 (ddd, 1H, J 3.4, 6.1, 10.6 Hz, OCH₂), 3.45 (ddd, 1H, J 3.4, 6.9, 13.3 Hz, CH₂N₃), 3.36 (ddd, 1H, J 3.6, 6.1, 13.3 Hz, CH₂N₃), 2.14, 2.12, 2.09, 2.07 (each s, each 3H, 4xCOCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 170.3, 169.9, 169.8, 169.4 (COCH₃), 105.4 (C-1), 81.1 (C-2), 80.2 (C-4), 76.2 (C-3), 69.1 (C-5), 66.5 (OCH₂), 62.3 (C-6), 50.3 (CH₂N₃), 20.6, 20.54, 20.48, 20.43 (4xCOCH₃).

3.1.18. Azidoethyl β-D-galactofuranoside (29)—Compound **28** (4.48 g, 10.7 mmol) was debenzoylated with 7N NH₃/MeOH (10 mL) as described for the preparation of **20**. Purification by column chromatography (CHCl₃ – MeOH, 5:1) afforded **29** (2.67 g, 98%) as an oil. FABMS (NBA): m/z 250 [M+H]⁺. Anal. calcd for C₈H₁₅N₃O₆ • 1.5 H₂O: C, 34.78; H, 6.57; N, 15.21. Found: C, 34.77; H, 6.59; N, 15.20. ¹H NMR (300 MHz, D₂O): 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, OCH₂), 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, OCH₂), 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, CH₂N₃). ¹³C NMR (75 MHz, D₂O): δ 107.6 (C-1), 83.0 (C-4), 81.3 (C-2), 76.9 (C-3), 70.9 (C-5), 66.9 (OCH₂), 63.0 (C-6), 50.6 (CH₂N₃).

3.1.19. Azidoethyl 5,6-isopropylepine-β-D-galactofuranoside (30)—To a solution of compound **29** (2.60 g, 10.4 mmol) in dry acetone (20 mL) was added 2,2'-dimethoxypropane (2.57 mL, 20.9 mmol) and (1S)-(+)-10-camphorsulfonic acid (242 mg, 1.04 mmol) at rt. After 1 h of stirring, the pH was adjusted to 7 by adding Et₃N. Concentration under vacuum gave the product which was dissolved in CHCl₃ (250 mL), washed with saturated NaHCO₃ (50 mL) and water (50 mL), dried over Na₂SO₄, and concentrated to an oil. Purification by column chromatography (CHCl₃ – MeOH, 99:1) yielded **30** (2.46 g, 82%) as an oil. FABMS (NBA): m/z 290 [M+H]⁺. Anal. Calcd for C₁₁H₁₉N₃O₆ • 0.5 H₂O: C, 44.29; H, 6.76; N, 14.09. Found: C, 44.29; H, 6.79; N, 14.01. ¹H NMR (300 MHz, CDCl₃): 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, OCH₂), 3.65 (m, 1H, OCH₂), 1.46 (m, 2H, CH₂N₃), 2.88 (d, 1H, J 11.6 Hz, 2-OH). ¹³C NMR (75 MHz, CDCl₃): 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 (OCH₂), 50.6 (CH₂N₃), 25.5, 25.9 (2xCH₃).

3.1.20. Azidoethyl 5,6-isopropylepine-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 CHCl₃ (200 mL). It was washed with water (50 mL), dried over Na₂SO₄, 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 C₂₇H₃₅N₃O₈: C, 61.24; H, 6.66; N, 7.93. Found: C, 61.11; H, 6.49; N, 7.77. ¹H NMR (300 MHz, CDCl₃): δ 7.29, 7.18 (m, each 2H, Ph), 6.89 (m, 4H, Ph), 5.07 (s, 1H, H-1), 4.44 (m, 4H, 2xCH₂Ph), 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, OCH₂), 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, 2xOCH₃), 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, OCH₂), 3.47 (m, 1H, CH₂N₃), 3.32 (m, 1H, CH₂N₃), 1.40, 1.35 (s, each 3H, 2xCH₃). ¹³C NMR (75 MHz, CDCl₃): δ: 159.4, 159.3 (2xC), 129.7 (CH), 129.3 (C), 113.8, 113.7 (2xCH), 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 (2xCH₂Ph), 66.3 (OCH₂), 65.3 (C-6), 55.2, 55.2 (2xOCH₃), 50.5 (CH₂N₃), 26.4, 25.4 (2xCH₃).

3.1.21. 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 with CHCl₃ (20 mL) and neutralized with saturated NaHCO₃ solution. The organic phase was washed with water (2x50 mL), dried over Na₂SO₄, and purified by column chromatography (CHCl₃ – MeOH, 95:5) to give **32** (1.78 g, 77%) as an oil. FABMS: *m/z* 507 [M+NH₄]⁺. Anal. calcd for C₂₄H₃₁N₃O₈ • 0.5 H₂O: C, 57.82; H, 6.47; N, 8.43. Found: C, 57.85; H, 6.47; N, 8.39. ¹H NMR (300 MHz, CDCl₃): 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, 2xCH₂Ph), 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, OCH₂), 3.81, 3.79 (s, each 3H, 2xOCH₃), 3.73 (m, 1H, H-5), 3.65 (m, 2H, H₂-6), 3.59 (m, 1H, OCH₂), 3.46 (m, 1H, CH₂N₃), 3.33 (m, 1H, CH₂N₃), 2.51 (d, 1H, *J* 7.0 Hz, 5-OH), 2.23 (dd, 1H, *J* 5.1, 7.3 Hz, 6-OH). ¹³C NMR (75 MHz, CDCl₃): δ: 159.4, 159.32 (2xC), 129.7, 129.5 (3xCH), 129.4, 129.1 (2xC), 113.8, 113.8 (2xCH), 106.6 (C-1), 87.1 (C-2), 83.0 (C-3), 82.2 (C-4), 71.9, 71.8 (2xCH₂Ph), 71.4 (C-5), 66.3 (OCH₂), 64.3 (C-6), 55.2, 55.2 (2xOCH₃), 50.5 (CH₂N₃).

3.1.22. 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, redissolved 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 C₃₂H₃₉N₃O₉ • 0.5 H₂O: C, 62.12; H, 6.52; N, 6.79. Found: C, 62.15; H, 6.53; N, 6.75. ¹H NMR (300 MHz, CDCl₃): δ 7.23 (m, 6H, Ph), 6.87 (m, 6H, Ph), 5.04 (s, 1H, H-1), 4.44 (m, 6H, 3xCH₂Ph), 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, OCH₂), 3.80, 3.795, 3.790 (each s, each 3H, 3xOCH₃), 3.57 (m, 1H, OCH₂), 3.48 (d, 2H, J 6.0 Hz, H₂-6), 3.43 (m, 1H, CH₂N₃), 3.31 (m, 1H, CH₂N₃), 2.40 (d, 1H, J 5.9 Hz, 5-OH). ¹³C NMR (75 MHz, CDCl₃) δ : 159.3, 159.2, 159.1 (3xC), 129.9, 129.6 (2xC), 129.6, 129.4, 129.2 (3xCH), 128.4 (C), 113.7, 113.67, 113.64 (3xCH), 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 (3xCH₂Ph), 69.9 (C-5), 66.2 (OCH₂), 55.15, 55.13, 55.12 (3xOCH₃), 50.4 (CH₂N₃).

3.1.23. 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 CHCl₃ (200 mL). The CHCl₃ layer was washed with water (2x50 mL), brine (2x50 mL) and dried over Na₂SO₄. 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 C₄₃H₄₅N₃O₈: C, 70.57; H, 6.20; N, 5.74. Found: C, 70.67; H, 6.23; N, 5.79. ¹H NMR (300 MHz, CDCl₃): 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, CH₂Ph), 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, OCH₂), 3.80, 3.79 (s, each 3H, 2xOCH₃), 3.54 (m, 1H, OCH₂), 3.40 (m, 1H, CH₂N₃), 3.26 (m, 2H, H-6a, CH₂N₃), 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). ¹³C NMR (75 MHz, CDCl₃) δ : 159.4, 159.2 (2xC), 143.8, 129.7 (2xC), 129.6, 129.4 (2xCH), 129.2 (C), 128.6, 127.7, 126.9 (3xCH), 113.8, 113.7 (2xCH), 106.5 (C-1), 87.2 (C-2), 86.6 (C), 83.0 (C-3), 81.7 (C-4), 71.9, 71.6 (2xCH₂Ph), 70.0 (C-5), 66.2 (OCH₂), 64.8 (C-6), 55.2 (OCH₃), 50.5 (CH₂N₃).

3.1.24. 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 redissolved in CHCl₃ (250 mL). It was washed with water (2x50 mL), dried over Na₂SO₄, 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 C₅₁H₅₃N₃O₉ • 0.15 H₂O: C, 72.28; H, 6.18; N, 4.86. Found: C, 72.25; H, 6.22; N, 4.89. ¹H NMR (300 MHz, CDCl₃): 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, 3xCH₂Ph), 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, OCH₃), 3.72 (m, 1H, OCH₂), 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, OCH₂), 3.35 (m, 2H, H-6a, CH₂N₃), 3.22 (m, 2H, H-6b, CH₂N₃). ¹³C NMR (75 MHz, CDCl₃) δ : 159.3, 159.19, 159.17 (3xC), 143.9, 130.3, 129.93 (3xC), 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 (3xCH₂Ph), 66.1 (OCH₂), 63.6 (C-6), 55.23, 55.20, 55.1 (3xOCH₃), 50.5 (CH₂N₃).

3.1.25. Azidoethyl 2,3,5-tri-*O*-*p*-methoxybenzyl- β -D-galactofuranoside (36)

Compound **35** (1.40 g, 1.64 mmol) in CHCl₃ (25 mL) was chilled to 0 °C. 5% TFA/CHCl₃ (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. NaHCO₃ and extracted with CHCl₃ (200 mL). The organic layer was washed with water (2x50 mL), dried over Na₂SO₄ 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 C₃₂H₃₉N₃O₉: C, 63.04; H, 6.30; N, 5.66. Found: C, 63.04; H, 6.30; N, 5.66.

6.45; N, 6.89. Found: C, 63.00; H, 6.42; N, 6.85. ^1H 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, $3\times\text{CH}_2\text{Ph}$), 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, $3\times\text{OCH}_3$), 3.64 (m, 4H, H-5, H-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 ($2\times\text{CH}$), 129.3, 129.2 ($2\times\text{C}$), 113.8, 113.7 ($2\times\text{CH}$), 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 ($3\times\text{CH}_2\text{Ph}$), 66.2 (OCH_2), 62.0 (C-6), 55.2, 55.1 ($3\times\text{OCH}_3$), 50.5 (CH_2N_3).

3.1.26. 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 $4\text{\AA}$ 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% $\text{Na}_2\text{S}_2\text{O}_3$ (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 $[\text{M}+\text{Li}]^+$. Anal. calcd for $\text{C}_{46}\text{H}_{57}\text{N}_3\text{O}_{18} \cdot 0.5 \text{H}_2\text{O}$: C, 58.22; H, 6.16; N, 4.23. Found C, 58.19; H, 6.19; N, 4.27. ^1H 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, $3\times\text{CH}_2\text{Ph}$), 3.83 (m, 1H, OCH_2), 3.80, 3.794, 3.793 (each s, each 3H, $3\times\text{OCH}_3$), 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 ($4\times\text{COCH}_3$), 159.4, 159.2, 159.1 ($3\times\text{C}$), 129.8, 129.3, 129.1, 113.8, 113.7, 113.6 ($12\times\text{CH}$), 72.9, 71.9, 71.7 ($3\times\text{CH}_2\text{Ph}$), 66.3 (OCH_2), 55.2, 55.2 ($3\times\text{OCH}_3$), 50.5 (CH_2N_3), 20.8, 20.7, 20.6, 20.5 ($4\times\text{COCH}_3$).

3.1.27. 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. $\text{H}_2\text{CO}_2\text{NH}_4$ (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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{46}\text{H}_{59}\text{NO}_{18} \cdot 0.5 \text{H}_2\text{O}$: C, 59.86; H, 6.55; N, 1.52. Found C, 59.90; H, 6.49; N, 1.48. ^1H 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, $3\times\text{CH}_2\text{Ph}$), 3.81 (s, 3H, OCH_3), 3.79 (s, 6H, $2\times\text{OCH}_3$), 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, $4\times\text{COCH}_3$). ^{13}C NMR (75 MHz, CDCl_3): non-sugar signals δ 170.4, 169.9, 169.9, 169.2 ($4\times\text{COCH}_3$), 159.3, 159.2, 159.0 ($4\times\text{C}$), 130.0, 129.7, 129.3 ($3\times\text{C}$), 129.6, 129.3, 129.0 ($3\times\text{CH}$), 113.7 ($2\times\text{CH}$), 113.6 (CH), 72.9 (CH_2Ph), 71.7 ($2\times\text{CH}_2\text{Ph}$), 69.2 (OCH_2), 63.0 (C-6'), 55.2 (OCH_3), 55.1 ($2\times\text{OCH}_3$), 41.9 (CH_2NH_2), 20.7, 20.6, 20.5, 20.4 ($4\times\text{COCH}_3$).

3.1.28. 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

CH₂Cl₂ (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 C₅₈H₇₀N₂O₂₀S • 0.5 H₂O: C, 60.25; H, 6.19; N, 2.42. Found C, 60.19; H, 6.18; N, 2.41. ¹H NMR (600 MHz, CDCl₃): 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, 3xCH₂Ph), 3.799, 3.795, 3.791 (each s, each 3H, 3xOCH₃), 3.59 (m, 1H, OCH₂), 3.40 (m, 1H, OCH₂), 3.07 (m, 2H, CH₂NH), 2.86 (s, 6H, N(CH₃)₂), 2.08, 2.06, 1.98, 1.94 (each s, each 3H, 4xCOCH₃). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals δ 170.5, 169.9, 169.2 (4xCOCH₃), 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 (CH₂Ph), 71.7 (2xCH₂Ph), 65.7 (OCH₂), 55.2, 55.2, 55.1 (3xOCH₃), 45.3 (N(CH₃)₂), 43.0 (CH₂NH), 20.8, 20.7, 20.6, 20.5 (4xCOCH₃).

3.1.29. 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*NH₃/MeOH (10 mL) overnight at rt. Concentration under vacuum followed by column chromatography (CHCl₃ – MeOH, 9:1) yielded **40** (285 mg, 98%) as an oil. FABMS (NBA): *m/z* 980 [M+H]⁺. Anal. calcd for C₅₀H₆₂N₂O₁₆S • 1.0 H₂O: C, 60.23; H, 6.47; N, 2.81. Found C, 60.19; H, 6.43; N, 2.84. ¹H NMR (600 MHz, CDCl₃): 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, 3xCH₂Ph), 3.79, 3.78, 3.76 (s, each 3H, 3xOCH₃), 3.56 (m, 2H, H-6'b, OCH₂), 3.36 (m, 1H, OCH₂), 3.03 (m, 2H, CH₂NH), 2.85 (s, 6H, N(CH₃)₂). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals δ 159.5, 159.3, 159.2 (3xC), 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 (10xCH), 73.0, 71.7, 71.6 (3xCH₂Ph), 65.3 (OCH₂), 55.3, 55.22, 55.20 (3xOCH₃), 45.3 (N(CH₃)₂), 42.8 (CH₂NH).

3.1.30. 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 CH₂Cl₂ (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 C₅₆H₆₄N₄O₂₀S • 0.5 H₂O: C, 58.28; H, 5.68; N, 4.85. Found C, 58.27; H, 5.59; N, 4.80. ¹H NMR (300 MHz, CDCl₃): 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, 3xCH₂Ph), 3.79 (m, 3xOCH₃), 3.59 (m, 1H, OCH₂), 3.40 (m, 1H, OCH₂), 3.07 (m, 2H, CH₂NH), 2.08, 2.06, 1.98, 1.94 (s, each 3H, 4xCOCH₃). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals δ 170.6, 170.0, 169.4 (4xCOCH₃), 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 (3xCH₂Ph), 65.7 (OCH₂), 55.3, 55.2 (3xOCH₃), 43.2 (CH₂NH), 20.8, 20.7, 20.6, 20.5 (4xCOCH₃).

3.1.31. 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 CH₂Cl₂ – H₂O (10:1) and DDQ (1.16 g, 5.1 mmol) was added at rt. The reaction mixture was stirred for 4 h, dried over Na₂SO₄ 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{32}\text{H}_{40}\text{N}_4\text{O}_{17}\text{S} \cdot 1.5 \text{H}_2\text{O}$: C, 47.35; H, 5.34; N, 6.90. Found C, 47.29; H, 5.33; N, 6.87. ^1H 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, $2\times\text{OCH}_3$). ^{13}C NMR (75 MHz, CDCl_3): non-sugar signals, δ 171.2, 170.4, 170.2 ($4\times\text{COCH}_3$), 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).

3.1.32. 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 $[\text{M}+\text{Li}]^+$. Anal. calcd for $\text{C}_{46}\text{H}_{57}\text{N}_3\text{O}_{18}$: C, 58.78; H, 6.11; N, 4.47. Found C, 58.76; H, 6.08; N, 4.43. ^1H 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, $3\times\text{CH}_2\text{Ph}$), 3.85 (m, 1H, OCH_2), 3.81, 3.764, 3.757 (s, each 3H, $3\times\text{OCH}_3$), 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, $4\times\text{COCH}_3$). ^{13}C NMR (75 MHz, CDCl_3): non-sugar signals, δ 170.5, 169.9, 169.5 ($4\times\text{COCH}_3$), 159.3, 159.22, 159.19 ($3\times\text{C}$), 130.2 (C), 129.8 (CH), 129.7 (C), 129.6 (CH), 129.5 (C), 113.7, 113.67, 113.61 ($16\times\text{CH}$), 73.0, 71.7, 71.6 ($3\times\text{CH}_2\text{Ph}$), 66.5 (OCH_2), 55.2, 55.20, 55.17 ($3\times\text{OCH}_3$), 50.5 (CH_2N_3), 20.8, 20.7, 20.6, 20.6 ($4\times\text{COCH}_3$).

3.1.23. 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. $\text{H}_2\text{CO}_2\text{NH}_4$ (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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{46}\text{H}_{59}\text{NO}_{18}$: C, 60.45; H, 6.51; N, 1.53. Found C, 60.48; H, 6.47; N, 1.54. ^1H 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, $3\times\text{OCH}_3$), 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, $4\times\text{COCH}_3$).

3.1.34. 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 $[\text{M}+\text{H}]^+$. Anal. calcd for $\text{C}_{58}\text{H}_{70}\text{N}_2\text{O}_{20}\text{S}$: 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, CH₂Ph), 3.81 (s, 3H, OCH₃), 3.77, 3.75 (each s, each 3H, 2xOCH₃), 3.61 (m, 1H, OCH₂), 3.35 (m, 1H, OCH₂), 3.04 (m, 2H, CH₂NH), 2.86 (s, 6H, N(CH₃)₂), 2.11, 2.09, 2.01, 2.01 (each s, each 3H, 4xCOCCH₃). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals, δ 170.5, 170.0, 169.9, 169.5 (4xCOCCH₃), 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 (3xCH₂Ph), 65.7 (OCH₂), 55.2, 55.2, 55.1 (3xOCH₃), 45.4 (NCH₃), 42.9 (CH₂NH), 20.8, 20.7, 20.6, 20.5 (4xCOCCH₃).

3.1.35. 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 CH₂Cl₂ (10 mL) was cooled to –78 °C under argon. SnCl₄ (0.05 mL, 0.40 mmol) was added and the mixture was stirred 1h at –78 °C and further overnight at –20 °C. A saturated NaHCO₃ solution (5 mL) was added and the reaction was further diluted with CHCl₃ (30 mL). The organic layer was washed with water (2x10 mL), dried over Na₂SO₄ and concentrated to an oil. Chromatography (CHCl₃ – MeOH, 95:5) yielded **46** (85 mg, 83%) as an oil. FABMS (NBA): *m/z* 787 [M+H]⁺. Anal. calcd for C₃₄H₄₆N₂O₁₇S • 1.0 H₂O: C, 50.74; H, 6.01; N, 3.48. Found: C, 50.77; H, 6.00; N, 3.44. ¹H NMR (300 MHz, CDCl₃, D₂O 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, OCH₂), 3.40 (m, 1H, OCH₂), 3.08 (dd, 2H, *J* 4.6, 5.4 Hz, CH₂NH), 2.89 (s, 6H, N(CH₃)₂), 2.14, 2.12, 2.07, 2.06 (each s, each 3H, 4xCOCCH₃). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals, δ 170.7, 170.3, 170.1, 170.0 (4xCOCCH₃), 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 (OCH₂), 43.00 (CH₂NH), 31.8, 29.4 (N(CH₃)₂), 20.8, 20.76, 20.72, 20.6 (4xCOCCH₃).

3.1.36. 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-naphthaenesulfonyl chloride (967 mg, 0.40 mmol) in dry CH₂Cl₂ (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 C₅₆H₆₄N₄O₂₀S: C, 58.73; H, 5.63; N, 4.89. Found C, 58.76; H, 5.69; N, 4.87. ¹H NMR (300 MHz, CDCl₃): 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, CH₂Ph), 3.82, 3.78, 3.76 (each s, each 3H, 3xOCH₃), 3.56 (m, 1H, OCH₂), 3.33 (m, 1H, OCH₂), 3.08 (m, 2H, CH₂NH), 2.12, 2.09, 2.02, 2.01 (each s, each 3H, 4xCOCCH₃). ¹³C NMR (75 MHz, CDCl₃): non-sugar signals, δ 170.6, 170.1, 170.00, 169.5 (COCCH₃), 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 (3xCH₂Ph), 65.6 (OCH₂), 55.2, 55.2, 55.1 (3xOCH₃), 43.0 (CH₂NH), 20.8, 20.7, 20.6, 20.5 (4xCOCCH₃).

3.1.37. 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 CH₂Cl₂ – H₂O (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 Na₂SO₄ and concentrated to syrup. Column chromatography (CHCl₃ – 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, $4 \times COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): non-sugar signals, δ 170.8, 170.4, 170.1, 17.1 ($\underline{COCH_3}$), 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 ($4 \times COCH_3$).

3.1.38. 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

3.1.39. 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-BeadsTM 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. 1H 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 (2xC), 131.2 (CH), 131.2, 130.9 (2xC), 130.0, 129.1, 124.3, 120.6, 116.5 (5xCH), 67.6 (OCH_2), 45.8 ($N(CH_3)_2$), 43.9 (CH_2NH).

3.1.40. 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-BeadsTM 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. 1H 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 (2xC), 131.2 (CH), 131.2, 130.9 (2xC), 130.0, 129.1, 124.3, 120.6, 116.5 (5xCH), 67.5 (OCH_2), 45.8 ($N(CH_3)_2$), 43.9 (CH_2NH).

3.1.41. 5-Azidonaphthalene-1-sulfonamidoethyl 5-*O*-(α -D-arabinofuranosyl)- α -D-arabinofuranoside (4)—**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 have been reported.¹⁴

3.1.42. 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 7N NH₃/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 – H₂O (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 C₂₄H₃₂N₄O₁₃S • 1.0 H₂O: C, 45.42; H, 5.40; N, 8.53. Found C, 45.49; H, 5.39; N, 8.61. ¹H NMR (600 MHz, CD₃OD): 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, OCH₂), 3.28 (m, 1H, OCH₂), 3.07 (t, 2H, *J* 5.6 Hz, CH₂NH). ¹³C NMR (75 MHz, CD₃OD): 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 (OCH₂), 43.9 (CH₂NH).

3.1.43. 5-Azidonaphthalene -1-sulfonamidoethyl 6-O-(β-D-galactofuranosyl)-β-D-galactofuranoside (6)—Disaccharide **48** (700 mg, 0.89 mmol) was deacetylated in 7N NH₃/MeOH (20 mL) as described for the preparation of **40**. An aqueous solution (5 mL) crude disaccharide was passed through a small column of Bio-Beads™ SM-4 (25 g, 20–50 mesh) and eluted with MeOH – H₂O (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 C₂₄H₃₂N₄O₁₃S • 1.0 H₂O: C, 45.42; H, 5.40; N, 8.53. Found C, 45.49; H, 5.41; N, 8.57. ¹H NMR (600 MHz, CD₃OD): 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, OCH₂), 3.29 (m, 1H, OCH₂), 3.08 (t, 2H, *J* 5.5 Hz, CH₂NH). ¹³C NMR (75 MHz, CD₃OD): 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 (OCH₂), 43.9 (CH₂NH).

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 were stored as 100 mM ethanol stocks and DP[¹⁴C]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 MgCl₂, 1 mM ATP, *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 CHCl₃:CH₃OH (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 C₂H₅OH:H₂O (1:1, 1 mL) and loaded onto a pre-equilibrated (C₂H₅OH:H₂O [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 H₂O (3 mL). The resulting organic phase was recovered following centrifugation at 3,500 × g and the aqueous phase was again extracted twice with 3 mL of *n*-butanol saturated water, the pooled extracts were back-washed twice with water saturated with *n*-butanol (3 mL). The *n*-butanol-saturated water fraction was dried and re-suspended in 200 μL of *n*-butanol. The total cpm of radiolabeled

material extractable into the *n*-butanol phase was measured by scintillation counting using 10 % of the labeled material and 10 mL of EcoScintA (National Diagnostics, Atlanta). The incorporation of [¹⁴C]Araf 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 CHCl₃:CH₃OH:NH₄OH:H₂O (65:25:0.5:3.6) on aluminum backed Silica Gel 60 F₂₅₄ plates (E. Merck, Darmstadt, Germany). Autoradiograms were obtained by exposing TLC's 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-[¹⁴C]Galp (Amersham, 257 mCi/mmol) in a buffer containing 50 mM MOPS, pH 7.9, 5 mM 2-mercaptoethanol, 10 mM MgCl₂, 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 hour 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, resuspended 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 were subjected to scintillation counting, and another 50 μL were 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 [¹⁴C]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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Figure 1.
Structure representation of *M. tuberculosis* arabinogalactan (AG) structure

Figure 2.
Prototypical arabinosyltransferase-catalyzed reaction.

Figure 3.
Prototypical galactosyltransferase-catalyzed reaction.

Figure 4.

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 $\alpha(1\rightarrow5)$ and $\beta(1\rightarrow2)$ arabinofuranosyltransferase activities as reported earlier²⁵ through the inclusion of **1** at the highest concentration (2 mM), mycobacterial membranes and [¹⁴C]-DPA. TLC/autoradiography was performed using chloroform:methanol: NH₄OH:H₂O (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. 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 $\text{CHCl}_3:\text{MeOH}:\text{NH}_4\text{OH}:\text{H}_2\text{O}$ (65:25:0.4:3.6) and products revealed through exposure to Kodak X-Omat film at -70°C for 3 days.

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.

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. 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 $\text{CHCl}_3\text{:MeOH:NH}_4\text{OH:H}_2\text{O}$ (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.

Scheme 1.

Reagents and Conditions: (a) $\text{Cl}(\text{CH}_2)_2\text{OH}$, SnCl_4 , CH_3CN , rt, 85%, (b) NaN_3 , DMF, 85 °C; 7N NH_3 – MeOH, rt, 70% in two steps, (c) BzCl , Py, -78°C – rt, 58%, (d) TBDMSCl , DMF, imidazole, 85 °C, 81%, (e) 7N NH_3 – MeOH, rt, 93%, (f) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 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) 7N NH_3 – MeOH, rt, 93%, (j) $\text{Et}_4\text{N}^+\text{F}^-$, 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, CH₂Cl₂, –20 °C, 91%, (d) HCO₂NH₄, MeOH, 5% Pd/C, rt, 87%, (e) 5-Azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH₂Cl₂, 0 °C, 81%, (f) 7N NH₃ – MeOH, rt, 87%, (g) Et₄N⁺F[–], THF, rt, 80%.

Scheme 3.

Reagents and Conditions: (a) HOCH₂CH₂Cl, NIS, Sn(OTf)₂, CH₂Cl₂, -20 °C – rt, 86%, (b) NaN₃, DMF, 70 °C, *quant.*, (c) 7N NH₃ – MeOH, rt, *quant.*, (d) 2,2-Dimethoxypropane, camphor sulfonic acid, acetone, rt, 82%, (e) *p*-methoxybenzyl chloride (MPMCl), NaH, DMF, 0 °C – rt, 98%, (f) 60% *aq.* AcOH, 60 °C, 77%, (g) Bu₂SnO, MPMCl, toluene, reflux – rt, 90%, (h) TrCl, DMAP, Py, rt – 50 °C, 80%, (i) MPMCl, NaH, THF, 0 °C – rt, 86%, (j) 5% TFA in CH₃Cl, 0 °C, 70%.

Scheme 4.

Reagents and Conditions: (a) **26**, NIS, triflic acid, CH₂Cl₂, -20 °C, 66%, (b) Ph₃P, benzene, H₂O, 50 °C, 85%, (c) Dansyl chloride, *N*-methylimidazole, CH₂Cl₂, 0 °C, 77%, (d) Azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH₂Cl₂, 0 °C, 68%, (e) 7N NH₃ – MeOH, rt, **40**: *quant.*, **42**: 92%, (f) SnCl₄, CH₂Cl₂, thiophenol, -78 to -20 °C, **2**: 33%, **5**: 78%.

Scheme 5.

Reagents & Conditions: (a) **26**, NIS, triflic acid, CH₂Cl₂, -20 °C, 43%, (b) Ph₃P, benzene, H₂O, 50 °C, 75%, (c) Dansyl chloride, *N*-methylimidazole, CH₂Cl₂, 0 °C, 60%, (d) Azidonaphthalenesulfonyl chloride, *N*-methylimidazole, CH₂Cl₂, 0 °C, 68%, (e) SnCl₄, CH₂Cl₂, thiophenol, -78 to -20 °C, **46**: 83%, **48**: 81%, (f) 7N NH₃ – MeOH, rt, **3**: quant., **6**: 80%.

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

Table 1

¹H NMR Chemical Shifts for Ara_f/Disaccharides

Compd	Unit	Shifts δ (ppm), J (Hz)					
		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	Araf	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)
	Araf'	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	Araf	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)
	Araf'	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	Araf	4.69 (d) (1.8)	4.04 (dd) (3.3)	3.91 (m) (<i>n.d.</i>)	3.98 (m) (4.6)	3.84 (dd) (10.9)	3.61 (dd) (3.5)
	Araf'	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	Araf	4.68 (d) (1.5)	3.92 (dd) (3.4)	3.77 (m) (<i>n.d.</i>)	3.99 (m) (<i>n.d.</i>)	3.77 (m) (10.8)	3.58 (dd) (3.2)
	Araf'	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	Araf	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)
	Araf'	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	Araf	4.82 (d) (1.4)	3.99 (m) (<i>n.d.</i>)	3.99 (m) (<i>n.d.</i>)	3.99 (m) (4.6)	3.82 (dd) (10.6)	3.60 (dd) (4.2)
	Araf'	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	Araf	4.66 (d) (1.9)	3.91 (m) (<i>n.d.</i>)	3.91 (m) (<i>n.d.</i>)	3.91 (m) (4.5)	3.78 (dd) (10.8)	3.51 (m) (<i>n.d.</i>)
	Araf'	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	Araf	4.58 (d) (1.8)	3.80 (dd) (4.1)	3.93 (dd) (6.3)	3.71 (m) (<i>n.d.</i>)	3.71 (m) (<i>n.d.</i>)	3.62 (m) (<i>n.d.</i>)
	Araf'	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	Araf	4.68 (d) (1.5)	3.92 (dd) (3.4)	3.77 (m) (<i>n.d.</i>)	3.99 (m) (<i>n.d.</i>)	3.77 (m) (10.7)	3.58 (dd) (3.2)
	Araf'	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	Araf	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)

Compd	Unit	Shifts δ (ppm), J (Hz)				
		H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5a}$)	H-5a ($J_{5a,5b}$)
	Arqf'	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

¹³C NMR Chemical Shifts for Araf Disaccharides.

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

Table 3

¹H NMR Chemical Shifts for Gal^fDisaccharides.

Shifts δ (ppm), J (Hz)								
Compd	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) [*]	3.98 [*] (dd) (3.0)	3.99 (m) (<i>n.d.</i>)	4.06 (m) (<i>n.d.</i>)	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) [*]	3.83 [*] (dd) (2.4)	3.97 (m) (<i>n.d.</i>)	3.97 (m) (<i>n.d.</i>)	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) (<i>n.d.</i>)	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) (<i>n.d.</i>)
41	Gal ^f	4.87 (d) (1.2)	3.79 (m) (<i>n.d.</i>)	3.98 (m) (<i>n.d.</i>)	3.98 (m) (<i>n.d.</i>)	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 [†] (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 [#] (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) (<i>n.d.</i>)	3.95 (m) (6.3)	4.04 (dd) (3.0)	3.61 (m) (<i>n.d.</i>)	3.76 (m) (10.4)	3.45 (dd) (5.0)
	Gal ^f ''	5.04 (s) (0.5)	5.07 [†] (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)

Shifts δ (ppm), J (Hz)						
Compd	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 ($J_{5,6b}$)
47	Gal f''	5.06 (s) (0)	5.04 (m) (<i>n.d.</i>)	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)
	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) (<i>n.d.</i>) 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) (<i>n.d.</i>)	4.14 (u) (<i>n.d.</i>)	4.07 (u) (<i>n.d.</i>)	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) (<i>n.d.</i>)	3.99 (m) (<i>n.d.</i>)	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 [§] (ddd) (5.4)	3.82 (m) (<i>n.d.</i>)	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 [§] (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) (<i>n.d.</i>)	3.84 (m) (6.4) 3.66 (dd) (11.8) 3.62 (m) (<i>n.d.</i>)
	Gal f''	5.15 (d) (1.5)	3.99 (m) (<i>n.d.</i>)	3.99 (m) (<i>n.d.</i>)	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)

* showed $J_{1,2} = 1.2$ Hz;

showed $J_{1,2} = 1.8$ Hz;

† showed $J_{1,2} = 0.5$ Hz;

§ showed $J_{1,3} = 0.5$ Hz;

n.d. = not determined due to complexity of signal, u = unresolved signal

Table 4

¹³C NMR Chemical Shifts for Gal^f Disaccharides.

Compd	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

Compd	Unit	Shifts δ (ppm)					
		C-1	C-2	C-3	C-4	C-5	C-6
47	Gal f'	105.7	81.1	76.5	80.1	69.3	62.8
	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