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Synthesis of mannopyranose disaccharides as photoaffinity probes for mannosyltransferases in *Mycobacterium tuberculosis*

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Abstract—Mannosyltransferases play a crucial role in mycobacterial cell-wall biosynthesis and are potential new drug targets for the treatment of tuberculosis. Herein, we describe the synthesis of α-(1→2)- and α-(1→6)-linked mannopyranosyl disaccharides possessing a 5-azidonaphthlene-1-sulfonamidoethyl group as photoaffinity probes for active-site labeling studies of mannosyltransferases in *Mycobacterium tuberculosis*.

Keywords: Mannose; Disaccharide; Photoaffinity probe; Mannosyltransferases; Mycobacterium tuberculosis

1. Introduction

Tuberculosis (TB) remains a major public health emergency in spite of the ready availability of antibiotics. This situation is exacerbated in the developing world and has raised concerns regarding vulnerability of developed nations, due to the widespread emergence of multi-drug resistant forms of the disease.[1] Recent advances in the knowledge of the biology of this organism and the availability of the genome sequence have provided insight into metabolism and persistence of the TB bacillus and point to a wide range of novel targets for new drug design.[2] The mycobacterial cell wall is a complex mixture of unique components that sets mycobacteria apart from other typical bacterial species and is a proven, effective target based on the known mechanism of action of other frontline agents.[2,3] Two important TB drugs, isoniazid and ethambutol, target mycobacterial cell-wall synthesis, specifically the biosynthesis of arabinogalactan and mycolic acids, respectively.[4] Lipoarabinomannan (LAM) is a major component of the mycobacterial cell envelope and is in large part responsible for the immunopathogenesis of tuberculosis. LAM is a sizeable cell-wall component known to cause abrogation of T-cell activation, inhibition of protein kinase C activity, and evocation of murine macrophages, inhibition of γ-interferon-mediated activation of murine macrophages etc. Both LAM and the structurally related lipomannan (LM) from *Mycobacterium tuberculosis* (Mtb) contain a phosphatidylinositol unit and an α-(1→6)-linked mannan core capped with α-(1→2)-linked mannoses.[5] GDP-mannose is the natural mannose donor utilized by mycobacterial mannosyltransferases to make LM/LAM, both of which are constructed through two mannosyl phosphidolipids, C_{35}-P-mannose and C_{50}-P-mannose.[6] It has been reported that the pimB gene of Mtb encodes a mannosyltransferase involved in LAM biosynthesis.[7] Very recently, a new gene encode, pimC, has been identified that encodes an α-mannosyltransferase involved in triacylphosphatidylinositol trimannoside.[8] α-Mannosyltransferases that are involved in the biosynthesis of phosphatidyl *myo*-inositol mannoside (PIM) and LM are prospective targets for the design of novel chemotherapeutic agents against pathogenic mycobacteria.

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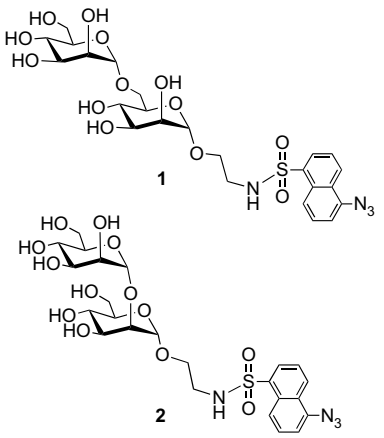


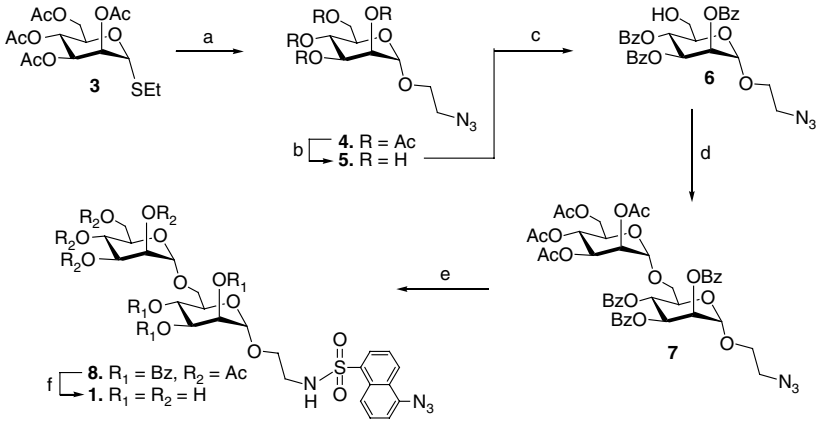
Figure 1. Synthetic probes for mannosyltransferases in Mtb.

Photoaffinity labeling of enzyme substrates by utilizing photoactivatable heterobifunctional aromatic azido reagents is useful for the identification of specific binding proteins and enzyme active sites.[9,10] Upon exposure to ultraviolet light, the azide decomposes into a reactive nitrene intermediate that can react with neighboring bonds to form a cross-link between the ligand and its binding protein. In continuation of our work preparing saccharide substrates and inhibitors for the study of mycobacterial glycosyltransferases,[11,12] we report herein the synthesis of two mannopyranose (Man*p*) disaccharide photoprobes for the identification of the specific mannosyltransferases and their substrate binding site(s). Binding and photoactivation of the enzyme–dissaccharide complex should fluorescently label the putative pim protein product at or near the enzyme active site. Similar to the reported synthesis of an Ara*f* disaccharide photoaffinity probe,[12] two α-(1→2)- and α-(1→6)-linked Man*p*–Man*p* disaccharides (Fig. 1, structures **1** and **2**) bearing an 5-azidonaphthalene-1-sulfonyl group at the reducing end were prepared successfully as potential mannosyl photoaffinity probes to identify putative mannosyltransferases involved in mycobacterial lipoarabinomannan (LAM) biosynthesis. Herein, we have used 5-azidonapthalene-1-sulfonyl chloride[10] as a heterobifunctional reagent (both a photocoupling moiety as well as a potential end-product fluorescent label) for coupling to the α-Man*p*-(1→6)-Man*p* and α-Man*p*-(1→2)-Man*p* disaccharides.

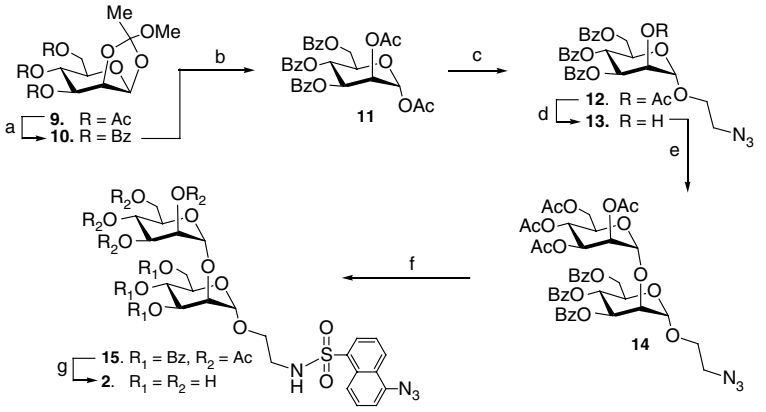
2. Results and discussion

2.1. Synthesis of mannose disaccharides as probes

The syntheses of the target photoaffinity probes 5-azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1**) and 5-azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (**2**) are presented in Schemes 1 and 2, respectively. For the synthesis of photoaffinity probe **1** (Scheme 1), the acceptor sugar **6** bearing an unblocked 6-OH group was synthesized in a reaction sequence starting from commercially available ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (**3**) that was coupled with the 1-azidoethanol[13] in the presence of *N*-iodosuccinimide (NIS) and the Lewis acid promoter triflic acid to give α-glycosylated sugar **4** in excellent yield for further selective protection and deprotection steps. Compound **4** was then deacetylated at ambient temperature using 7 N ammonia in methanol to produce compound **5**. The OH group at the 6-position in compound **5** was blocked by a trityl group using trityl chloride in pyridine at 40 °C, and after 48 h, the reaction mixture was cooled to room temperature. To the mixture was added benzoyl chloride, and the reaction was stirred for another 12 h at room temperature. After the usual workup, the crude 1-azidoethyl 2,3,5-tri-*O*-benzoyl-6-*O*-trityl-α-D-mannopyranose obtained was detritylated using 5% trifluoroacetic acid in chloroform at 0 °C in 4 h. Pure compound **6** was obtained in 86% overall yield after purification by column chromatography. Coupling of acceptor **6** and donor **3** was carried out in the presence of NIS and the promotor, triflic acid. Addition of the reagents and the subsequent reaction was carried out at −20 °C under an argon atmosphere in dry CH_2Cl_2 over powdered 4 Å molecular sieves. These conditions stereospecifically afforded the disaccharide **7** in 77% yield after silica gel column chromatography. The structure of disaccharide **7** was unambiguously confirmed by MS and NMR spectral data. The characteristic resonances due to both anomeric protons H-1 and H-1′ were located as a doublets at 5.13 ppm with $J_{1,2} = 1.5$ Hz and at 4.87 ppm with $J_{1',2'} = 1.7$ Hz, respectively, in the ^{1}H NMR spectrum, whereas in the ^{13}C NMR spectrum C-1 and C-1′ were observed at 97.71 and 97.23 ppm, respectively, clearly indicated the 1,2-*trans* glycosylation. The azido group in disaccharide **7** was next reduced by reaction with HCO_2NH_4 in methanol using 5% Pd/C as catalyst for 4 h at room temperature, followed by rapid filtration and workup. The relative instability of **7** necessitated immediate reaction of the crude disaccharide bearing the amino functionality with 5-azidonaphthalene-1-sulfonyl chloride[10] in the presence of *N*-methylimidazole at 0 °C for 12 h to afford disaccharide **8**. Finally, compound **8** was deacylated using 7 N ammonia in methanol to give the target disaccharide 5-azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**1**) in 71% yield after column chromatography on silica gel, followed by lyophilization. The α-(1→6) linkage was supported by NMR spectral analysis as the ^{1}H NMR spectrum showed anomeric protons H-1′ and H-1 as doublets at 4.75 ppm with $J_{1',2'} = 1.7$ Hz and 4.39 ppm with



Scheme 1. (a) 1-Azidoethanol, NIS, triflic acid, CH_2Cl_2, −20 °C, 15 min, 71%; (b) 7 N NH_3–MeOH, rt, 12 h, 76%; (c) (1) TrCl, DMAP, Py, 40 °C, 48 h, (2) BzCl, Py, rt, 12 h; (3) 5% TFA–$CHCl_3$, $CHCl_3$, 0 °C, 4 h, 86%; (d) **3**, NIS, triflic acid, CH_2Cl_2, −20 °C, 15 min, 77%; (e) (1) HCO_2NH_4, 5% Pd/C, MeOH, rt, 4 h, (2) 5-azidonaphthalene-1-sulfonyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 12 h, 40%; (f) 7 N NH_3–MeOH, rt, 12 h, 71%.



Scheme 2. (a) (1) 7 N NH_3–MeOH, rt, 2 h, (2) BzCl, Py, rt, 3 h, 92%; (b) (1) 50% TFA–H_2O, CH_3CN, 2 h, (2) Ac_2O, Py, rt, 2 h, 78%; (c) 1-azidoethanol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 12 h, 76%; (d) 0.5% HCl in MeOH, rt, 72 h, 84%; (e) **3**, NIS, $Sn(OTf)_2$, CH_2Cl_2, −20 °C, 30 min, 67%; (f) (1) HCO_2NH_4, 5% Pd/C, MeOH, rt, 45 min, (2) 5-azidonaphthalene-1-sulfonyl chloride, *N*-methylimidazole, CH_2Cl_2, 0 °C, 12 h, 61%; (g) 7 N NH_3–MeOH, rt, 12 h, 81%.

$J_{1,2} = 1.4$ Hz, and the anomeric carbons C-1′ and C-1 appeared at 101.72 and 101.37 ppm, respectively, in ^{13}C NMR spectrum.

The synthesis of photoaffinity probe **2** (Scheme 2), an α-(1→2)-linked Man*p* disaccharide bearing an photoactivable group at the anomeric center, was carried out using the commercial 3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (**9**). First, compound **9** was deacetylated with 7 N NH_3–MeOH at room temperature and benzoylated with benzoyl chloride in pyridine at room temperature in one step to afford compound **10** in 92% yield. The 1,2-orthoester in compound **10** was opened at room temperature using 50% trifluoroacetic acid in water, similar to a reported procedure[14] to give 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranose that was further acetylated without purification by the reaction with acetic anhydride in pyridine at room temperature. Purification on a silica gel column afforded compound **11**, and the spectral data were compared with literature values.[15] The acceptor sugar **13**, containing a free hydroxyl group at the 2-position, was conveniently prepared from compound **11** via compound **12**. In a simple reaction sequence, 1-azidoethanol[13] was stereospecifically coupled with compound **11** using the Lewis acid $BF_3{\cdot}Et_2O$ in dichloromethane at room temperature in 12 h. The usual workup and column chromatography produced pure **12** in 76% yield. Deacetylation of compound **12** was achieved using 0.5% HCl in methanol at room temperature. After 72 h, thin-layer chromatography (TLC) showed complete reaction. Workup and column chromatography produced the acceptor sugar **13** in 84% yield. The disaccharide **14** was synthesized by the coupling reaction between donor sugar **3** and acceptor **13** using NIS and the promotor $Sn(OTf)_2$ at −20 °C in dichloromethane. TLC showed complete reaction in 30 min, followed by the usual workup and column chromatography to give disaccharide **14** in 67% yield.

The ^{1}H NMR spectrum showed the anomeric protons H-1 and H-1′ at 5.13 ppm ($J_{1,2} = 1.8$ Hz) and 4.96 ppm ($J_{1',2'} = 1.4$ Hz) as doublets, and in the ^{13}C NMR spectrum the anomeric carbons C-1 and C-1′ presented at 98.52 and 99.31 ppm, respectively. These data confirmed the α-(1 → 2)-linkage in the disaccharide. Similar to the earlier reduction procedure, disaccharide **14** bearing an azidoethyl group at the anomeric center of the reducing sugar was hydrogenated using HCO_2NH_4 and 5% Pd/C in methanol at room temperature. The crude amino disaccharide, obtained after a quick workup, was further reacted with 5-azidonaphthalene-1-sulfonyl chloride in presence of *N*-methylimidazole at 0 °C in dichloromethane for 12 h, and pure disaccharide **15** was obtained in 61% yield after column chromatography. The final α-(1 → 2)-linked mannopyranosyl disaccharide **2**, the desired photoaffinity probe, was obtained by deacylation of disaccharide **15** using 7 N NH_3–MeOH at ambient temperature. After column chromatography and lyophilization, pure 5-azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranoside (**2**) was obtained in 81% yield. The NMR spectrum showed the signals pertaining to the naphthyl group and the anomeric proton H-1′ appeared as a doublet at 4.89 ppm with $J_{1',2'} = 1.4$ Hz, whereas the H-1 appeared at 4.86 ppm as an unresolved multiplet. The structure of disaccharide **2** was also confirmed by the ^{13}C NMR spectrum that showed the anomeric carbons C-1 at 99.94 ppm and C-1′ at 104.10 ppm with the other expected signals.

2.2. Acceptor activity of mannose disaccharides

Based on the previous use of specific mannose-based neoglycolipid acceptors,[16] compounds **1** (SRI 20723) and **2** (SRI 20731) were synthesized and compared as potential acceptors of [^{14}C]Man*p* from DP-[^{14}C]mannose generated in situ from GDP-[U-^{14}C]mannose within a mannosyltransferase assay. Assays performed in the presence of membranes (*M. smegmatis* for compound **1** and *M. Bovis* for compound **2**) and compound **1** resulted in [^{14}C]Man*p* incorporation from DP-[^{14}C]mannose (Figs. 2 and 3). The related compound **2** possessed no detectable activity.

In conclusion, highly effective and concise syntheses of photoaffinity probes **1** and **2** were achieved stereoselectively. However only the (1 → 6)-linked Man*p* disaccharide showed acceptor capability in a cell-free mannosyltransferase assay. All new compounds were characterized by ESIMS and NMR spectroscopy. CHN analyses were performed on the target compounds. The NOE, decoupling, D_2O exchanged and APT experiments were performed as required in order to confirm NMR assignments and stereochemistry at the anomeric center of sugars.

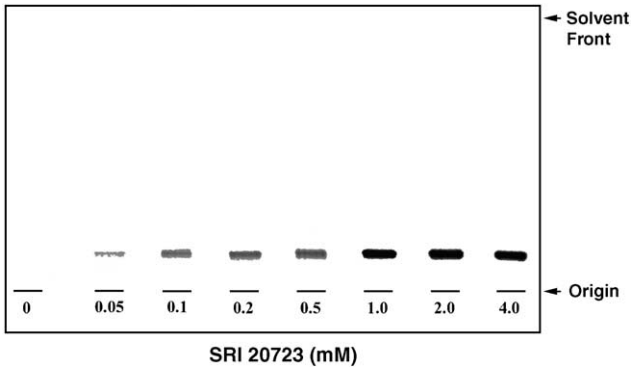


Figure 2. An autoradiogram of reaction products produced through the inclusion of acceptor **1**.

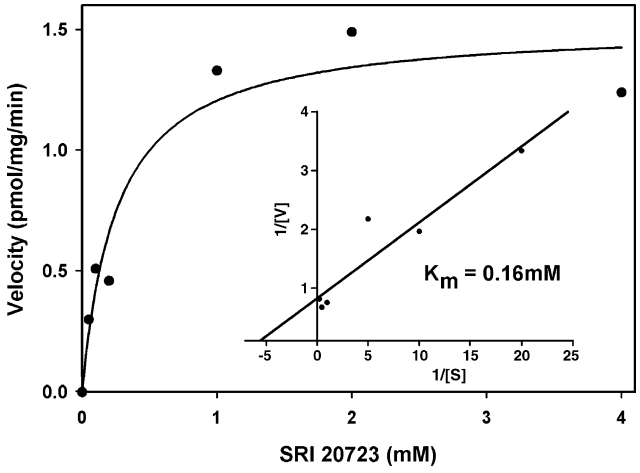


Figure 3. Kinetic analysis of acceptor **1**. The insert illustrates the double reciprocal plot for **1** as a substrate for the mycobacterial mannosyltransferase.

3. Experimental

3.1. Synthesis

3.1.1. General procedures. All manipulations were conducted under a dry argon atmosphere. Reaction temperatures were measured externally. Anhydrous solvents from Aldrich Chemical Co. were used without further drying. Whenever necessary, compounds were dried by azeotropic removal of water with toluene under reduced pressure. The starting materials, ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranoside (**3**) and 3,4,6-tri-*O*-acetyl-1,2-*O*-(methoxyethylidene)-β-D-mannopyranose (**9**) were purchased from Toronto Research Chemicals, Inc. (www.trc-canada.com) and used as received. Reactions were monitored by TLC on pre-coated E. Merck silica gel ($60F_{254}$) plates (0.25 mm) and visualized using UV light (254 nm) and/or heating after spraying with $(NH_4)_2SO_4$ solution (150 g ammonium sulfate, 30 mL H_2SO_4, 750 mL H_2O). All solvents used for workup and chromatography were reagent grade from

Fisher Scientific Co. Column chromatography was carried out on Fischer brand silica gel 60 (230–400 mesh). All reactions involving aryl azides were carefully performed under a minimum of ambient light to ensure stability of the reactants, and these materials were carefully kept from light in the freezer during storage. Melting points were determined with a Mel-Temp II capillary melting points apparatus and uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on Nicolet NT 300NB instrument at 300 and 75 MHz, respectively. Certain ^{1}H NMR spectra were recorded on a Bruker Advance 600 System at 600 MHz. The coupling constants (J) are reported in Hz, and chemical shifts are reported in ppm (δ) relative to residual solvent peak or internal standard. Microanalyses were performed on a Perkin–Elmer 2400 CHN analyzer. ESIMS was recorded on a BioTof-2 time-of-flight mass spectrometer.

3.1.2. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (4). Compound **3** (2.00 g, 5.10 mmol) and activated, powdered 4 Å molecular sieves (2.00 g) in dry CH_2Cl_2 (60 mL) were cooled to −20 °C. The 1-azidoethanol (0.6 mL, 6.12 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise under argon atmosphere. The mixture was stirred for 15 min, and NIS (2.30 g, 10.2 mmol), followed by triflic acid (45 μL, 0.51 mmol), were added to initiate coupling. The reaction mixture was allowed to stir for 30 min at rt, and the reaction was quenched by addition of Et_3N (1 mL), diluted with CH_2Cl_2 (50 mL), and filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (20 mL), followed by washing with satd aq $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, the solvent was removed in vacuo, and flash chromatography (3:1 $CHCl_3$–MeOH) afforded **4** (1.52 g, 71%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3), 5.30 (dd, 1H, $J_{3,4}$ 9.9 Hz, $J_{4,5}$ 9.5 Hz, H-4), 5.28 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2), 4.87 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.29 (dd, 1H, $J_{5,6a}$ 5.3 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.13 (dd, 1H, $J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 12.2 Hz, H-5b), 4.05 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6a}$ 5.3 Hz, $J_{5,6b}$ 2.4 Hz, H-5), 3.91–3.84 (m, 1H, OCH_2), 3.71–3.64 (m, 1H, OCH_2), 3.50–3.45 (m, 2H, CH_2N_3), 2.17, 2.11, 2.06, 2.00 (s, each 3H, 4×OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.49, 169.89, 169.69, 169.64 (C), 97.65 (C-1), 69.30 (C-2), 68.77 (C-3, C-5), 66.95 (OCH_2), 65.93 (C-4), 62.37 (C-6), 50.27 (CH_2N_3), 20.76, 20.64, 20.61, 20.55 (OCH_3). ESIMS: m/z 440.1293 $(M + Na)^+$, calcd for $C_{16}H_{23}N_3O_{10}$ 440.1275 $(M + Na)^+$.

3.1.3. 2-Azidoethyl α-D-mannopyranoside (5). To a solution of compound **4** (1.5 g, 3.59 mmol) in dry MeOH (10 mL), was added 7 N NH_3–MeOH (20 mL) dropwise, and the reaction mixture was stirred at room temperature for 12 h. Concentration in vacuo and flash chromatography (3:1 $CHCl_3$–MeOH) gave **5** as a solid (678 mg, 76%): mp 142 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 4.80 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 3.95–3.88 (m, 1H, OCH_2), 3.86–3.82 (m, 2H, H-2, H-5), 3.74–3.56 (m, 5H, H-3, H-4, H_2-6, OCH_2), 3.42–3.39 (m, 2H, CH_2N_3). ^{13}C NMR (75 MHz, CD_3OD): δ 101.80 (C-1, $^1J_{C,H}$ 168.7 Hz), 74.89 (C-5), 72.47 (C-3), 72.05 (C-2), 68.53 (C-4), 67.69 (OCH_2), 62.93 (C-6), 51.73 (CH_2N_3). ESIMS: m/z 272.0853 $(M + Na)^+$, calcd for $C_8H_{15}N_3O_6$ 272.0853 $(M + Na)^+$.

3.1.4. 2-Azidoethyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (6). Compound **5** (500 mg, 2.01 mmol) was dissolved in dry pyridine (10 mL), and to it was added trityl chloride (671 mg, 2.41 mmol) and DMAP (25 mg, 0.20 mmol) under an argon atmosphere. The reaction mixture was stirred at 40 °C for 48 h and cooled to room temperature. Benzoyl chloride (0.80 mL, 6.55 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The mixture was poured into an ice-water mixture and extracted with $CHCl_3$ (2×100 mL). The $CHCl_3$ layer was dried over Na_2SO_4, concentrated, and dried in vacuo overnight. The resulting syrup was dissolved in $CHCl_3$ (10 mL), cooled to 0 °C, and to it was added 5% trifluoroacetic acid in $CHCl_3$ (10 mL). The reaction mixture was stirred at 0 °C for 4 h, and TLC showed the completion of the reaction. The mixture was poured into an ice-water mixture and extracted with $CHCl_3$ (2×75 mL). The organic layer was washed with satd aq $NaHCO_3$ (2×25 mL) and brine (25 mL) and finally dried over Na_2SO_4. After concentration, column chromatography over silica gel G (3:1 cyclohexane–AcOEt) afforded pure acceptor sugar **6** as an oil (842 mg, 86% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–8.08, 8.00–7.97, 7.84–7.81 (m, each 2H, Ar), 7.64–7.37 (m, 8H, Ar), 7.28–7.23 (m, 2H, Ar), 6.02 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.86 (t, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.71 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.4 Hz, H-2), 5.17 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.13 (ddd, 1H, $J_{4,5}$ 10.1 Hz, $J_{5,6a}$ 2.2 Hz, $J_{5,6b}$ 4.2 Hz, H-5), 4.04–3.97 (m, 1H, OCH_2), 3.86 (dd, 1H, $J_{5,6a}$ 2.2 Hz, $J_{6a,6b}$ 12.8 Hz, H-6a), 3.82–3.75 (m, 2H, H-6b, OCH_2), 3.64–3.56 (m, 1H, CH_2N_3), 3.52–3.45 (m, 1H, CH_2N_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.52, 165.49, 165.37 (C=O), 133.67, 133.58, 133.16 (CH), 130.11 (C), 129.91, 129.67 (CH), 129.17, 129.06 (C), 128.62, 128.49 (CH), 128.26 (CH), 97.83 (C-1), 71.31 (C-5), 70.40 (C-2), 69.37 (C-3), 67.20 (OCH_2), 67.07 (C-4), 61.29 (C-6), 50.41 (CH_2N_3). ESIMS: m/z 584.1659 $(M + Na)^+$, calcd for $C_{29}H_{27}N_3O_9$ 584.1639 $(M + Na)^+$.

3.1.5. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (7). The glycosylation reaction was carried out by the reaction of alcohol **6** (760 mg,1.35 mmol) and glycosylation donor **3** (797 mg, 2.03 mmol) in the presence of NIS (608 mg, 2.7 mmol), triflic acid (10 μL, 0.14 mmol),

and powdered 4 Å molecular sieves (500 mg) in dry CH_2Cl_2 (10 mL) at −20 °C as described for the synthesis of disaccharide **4**. Purification by column chromatography (2:1 cyclohexane–AcOEt) yielded disaccharide **7** (832 mg, 77%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13–8.10, 7.99–7.96, 7.83–7.80 (m, each 2H, Ar), 7.65–7.59, 7.55–7.50, 7.44–7.36, 7.28–7.22 (m, 9H, Ar), 5.91–5.85 (m, 2H, H-3, H-4), 5.73 (dd, 1H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.0 Hz, H-2), 5.41 (dd, 1H, $J_{2',3'}$ 3.4 Hz, $J_{3',4'}$ 10.1 Hz, H-3′), 5.30–5.23 (m, 2H, H-2′, H-4′), 5.13 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.87 (d, 1H, $J_{1',2'}$ 1.7 Hz, H-1′), 4.40–4.35 (m, 1H, H-5), 4.16–4.08 (m, 1H, OCH_2), 4.06–3.93 (m, 4H, H-6a, H-5′, H_2-6′), 3.83–3.76 (m, 1H, OCH_2), 3.66 (dd, 1H, $J_{5,6b}$ 1.9 Hz, $J_{6a,6b}$ 10.8 Hz, H-6b), 3.63–3.52 (m, 2H, CH_2N_3), 2.13, 2.06, 2.00, 1.94 (s, each 3H, 4×OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.52, 169.89, 169.71, 169.62 (C═O), 165.57, 165.54, 165.36 (C═O), 133.60, 133.57, 133.15 (CH), 129.93, 129.84, 129.73 (CH), 129.20, 129.02, 128.79 (C), 128.73, 128.52, 128.27 (CH), 97.71 (C-1), 97.23 (C-1′), 70.42 (C-2), 69.83, 69.65, 69.49, 68.92, 68.71 (C-3, C-5, C-2′, C-3′, C-4′), 67.16 (OCH_2), 66.94 (C-4), 66.34 (C-6), 65.96 (C-5′), 62.31 (C-6′), 50.47 (CH_2N_3), 20.83, 20.74, 20.69, 20.57 (OCH_3). ESIMS: m/z 914.2616 $(M + Na)^+$, calcd for $C_{43}H_{45}N_3O_{18}$ 914.2590 $(M + Na)^+$.

3.1.6. 5-Azidonaphthalene-1-sulfonamidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (8). Compound **7** (720 mg, 0.81 mmol) was dissolved in dry MeOH (20 mL), and 5% Pd/C (500 mg) was added under an argon atmosphere. The HCO_2NH_4 (204 mg, 3.83 mmol) was added, and the reaction mixture was stirred at rt for 45 min, filtered through a short Celite pad, and concentrated. The resulting syrup was dissolved in $CHCl_3$ (20 mL), washed with water (2×10 mL), dried over Na_2SO_4, and concentrated. The crude, reduced disaccharide (620 mg, 0.83 mmol) was dissolved in dry CH_2Cl_2 (20 mL), and *N*-methyl imidazole (0.14 mL, 1.66 mmol) was added at 0 °C. To this cold solution was added 5-azidonaphthalene-1-sulfonyl chloride (334 mg, 1.25 mmol) under darkness to prevent photodecomposition. The reaction mixture was stirred for 12 h at 0 °C and quenched with water (10 mL). The organic layer was washed with water (2×10 mL), dried over Na_2SO_4, and concentrated. The pure product **8** was obtained after column chromatography (2:1 cyclohexane–AcOEt) as a light-sensitive oil (300 mg, 40%) that was stored in a dark, dry place until needed. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, 1H, *J* 8.7 Hz, Ar), 8.43 (dd, 1H, *J* 0.9, 8.5 Hz, Ar), 8.37 (dd, 1H, *J* 1.2, 7.3 Hz, Ar), 8.09–8.06, 7.96–7.93 (m, each 2H, Ar), 7.81–7.23 (m, 14H, Ar), 5.75–5.69 (m, 2H, H-3, H-4), 5.54 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 2.7 Hz, H-2), 5.36 (dd, 1H, $J_{2',3'}$ 3.2 Hz, $J_{3',4'}$ 10.1 Hz, H-3′), 5.27 (dd, 1H, $J_{3'4'}$ 10.1 Hz, $J_{4',5'}$ 9.5 Hz, H-4′), 5.22 (dd, 1H, $J_{1',2'}$ 1.8 Hz, $J_{2'3'}$ 3.2 Hz, H-2′), 4.83 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.82 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.23–4.19 (m, 1H, H-5), 4.17–4.01 (m, 3H, H-5′, H_2-6′), 3.91 (dd, 1H, $J_{5,6a}$ 7.4 Hz, $J_{6a,6b}$ 10.6 Hz, H-6a), 3.86–3.78 (m, 1H, OCH_2), 3.57 (dd, 1H, $J_{5,6b}$ 1.7 Hz, $J_{6a,6b}$ 10.6 Hz, H-6b), 3.53–3.48 (m, 1H, OCH_2), 3.33–3.19 (m, 2H, CH_2NH), 2.13, 2.08, 2.06, 1.96 (s, each 3H, 4×OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.50, 170.43, 170.03, 169.81 (C═O), 165.58, 165.34 (C═O), 137.68, 134.92 (C), 133.64, 133.23, 130.55, 129.87, 129.80, 129.68 (CH), 129.18, 129.11, 128.91 (C), 128.72 (CH), 128.68 (C), 128.63, 128.51, 128.29 (CH), 127.23 (C), 124.45, 120.96, 114.89 (CH), 97.59 (C-1), 96.86 (C-1′), 70.20 (C-2), 69.80, 69.76, 69.48, 69.16, 68.80 (C-3, C-5, C-2′, C-3′, C-4′), 66.95 (OCH_2), 66.64 (C-4), 66.16 (C-6), 65.74 (C-5′), 62.31 (C-6′), 42.41 (CH_2NH), 20.85, 20.81, 20.77, 20.58 (OCH_3). ESIMS: m/z 1119.2842 $(M + Na)^+$, calcd for $C_{53}H_{52}N_4O_{20}S$ 1119.2787 $(M + Na)^+$.

3.1.7. 3,4,6-Tri-*O*-benzoyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (10). To a dry MeOH (5 mL) solution of starting material **9** (750 mg, 2.07 mmol) was added 7 N NH_3–MeOH (15 mL), and the reaction mixture was stirred at rt for 2 h. The reaction mixture was concentrated in vacuo to a syrup that was further used for the benzoylation reaction without purification. The syrup was dissolved in dry pyridine (10 mL) under an argon atmosphere, and to it was added benzoyl chloride (900 μL, 7.80 mmol). The reaction mixture was stirred for 3 h at rt and poured into an ice-water mixture (50 mL). The mixture was extracted with $CHCl_3$ (2×50 mL) and concentrated to syrup. After purification on column chromatography (3:1 cyclohexane–AcOEt), the pure compound **10** was obtained as a colorless solid (1.04 g, 92%): mp 122–123 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04–7.99 (m, 4H, Ar), 7.93–7.90 (m, 2H, Ar), 7.56–7.47 (m, 3H, Ar), 7.42–7.32 (m, 6H, Ar), 5.91 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.66 (d, 1H, $J_{1,2}$ 2.6 Hz, H-1), 5.56 (dd, 1H, $J_{2,3}$ 3.9 Hz, $J_{3,4}$ 9.8 Hz, H-3), 4.86 (dd, 1H, $J_{1,2}$ 2.6 Hz, $J_{2,3}$ 3.9 Hz, H-2), 4.63 (dd, 1H, $J_{5,6a}$ 3.2 Hz, $J_{6a,6b}$ 12.0 Hz, H-6a), 4.46 (dd, 1H, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 12.0 Hz, H-6b), 4.07 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6a}$ 3.2 Hz, $J_{5,6b}$ 4.7 Hz, H-5), 3.27 (s, 3H, OCH_3), 1.77 (s, 3H, CH_3). ESIMS: m/z 571.1591 $(M + Na)^+$, calcd for $C_{29}H_{27}N_3O_9$ 571.1574 $(M + Na)^+$.

3.1.8. 2-Azidoethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (12). The 1,2-di-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranose (**11**) (854 mg, 1.48 mmol) was dissolved in dry CH_2Cl_2 (5 mL) and cooled to 0 °C, and 1-azidoethanol (193 mg, 2.22 mmol) was added under argon atmosphere. $BF_3{\cdot}Et_2O$ (750 μL, 5.92 mmol) was added dropwise, and the reaction mixture was stirred at room temperature, neutralized after 30 min with satd aq $NaHCO_3$ (10 mL), and diluted with CH_2Cl_2 (20 mL). After washing with water (10 mL) and brine (10 mL), the organic layer was dried over Na_2SO_4 and

evaporated. The crude syrup was purified by column chromatography (2:1 cyclohexane–AcOEt) to afford **12** (681 mg, 76%) as a colorless solid: mp 41–43 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07–8.04 (m, 2H, Ar), 7.97–7.88 (m, 4H, Ar), 7.58–7.46 (m, 3H, Ar), 7.44–7.32 (m, 6H, Ar), 5.93 (dd, 1H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.81 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3), 5.50 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.00 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.63 (dd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.50 (dd, 1H, $J_{5,6b}$ 5.2 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.42 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6a}$ 2.8 Hz, $J_{5,6b}$ 5.2 Hz, H-5), 4.01–3.94 (m, 1H, OCH_2), 3.78–3.71 (m, 1H, OCH_2), 3.62–3.54 (m, 1H, CH_2N_3), 3.50–3.42 (m, 1H, CH_2N_3), 2.13 (s, 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.80, 166.07, 165.50, 165.34 (C=O), 133.43, 133.24, 133.09, 129.82 (CH), 129.74 (C), 129.67, 129.64 (CH), 129.11, 128.87 (C), 128.41, 128.35 (CH), 97.61 (C-1), 69.75 (C-2), 69.58, 69.18 (C-3, C-5), 67.19 (C-6), 66.89 (C-4), 63.17 (OCH_2), 50.38 (CH_2N_3), 20.72 (OCH_3). ESIMS: m/z 626.1752 $(M + Na)^+$, calcd for $C_{31}H_{29}N_3O_{10}$ 626.1745 $(M + Na)^+$.

3.1.9. 2-Azidoethyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (13). To a CH_3CN (2 mL) solution of compound **12** (650 mg, 1.07 mmol) was added 0.5% HCl in MeOH (20 mL). The reaction mixture was stirred 72 h at rt and concentrated to near dryness. It was diluted with $CHCl_3$ (20 mL), washed with satd aq $NaHCO_3$ (20 mL) and water (20 mL). The organic layer was dried over Na_2SO_4 and concentrated to syrup. The product was further purified by flash chromatography (2:1 cyclohexane–AcOEt) to give **13** as colorless oil (530 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03–7.93 (m, 6H, Ar), 7.56–7.46 (m, 3H, Ar), 7.42–7.32 (m, 6H, Ar), 5.96 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.70 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.04 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.60 (dd, 1H, $J_{5,6a}$ 3.0 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.50 (dd, 1H, $J_{5,6b}$ 5.2 Hz, $J_{6a,6b}$ 11.9 Hz, H-6b), 4.43–4.37 (m, 2H, H-2, H-5), 4.03–3.96 (m, 1H, OCH_2), 3.78–3.71 (m, 1H, OCH_2), 3.53–3.48 (m, 2H, CH_2N_3), 2.39 (br s, 1H, 2-OH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.19, 165.53 (C=O), 133.37, 133.34, 133.04, 129.81 (CH), 129.72 (C), 129.68 (CH), 129.12, 129.03 (C), 128.44, 128.38, 128.37 (CH), 99.80 (C-1), 72.41 (C-2), 69.26, 69.07 (C-3, C-5), 67.07 (C-6), 66.85 (C-4), 63.43 (OCH_2), 50.41 (CH_2N_3). ESIMS: m/z 584.1657 $(M + Na)^+$, calcd for $C_{29}H_{27}N_3O_9$ 584.1639 $(M + Na)^+$.

3.1.10. 2-Azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (14). Compound **13** (675 mg,1.20 mmol) and activated, powdered 4 Å molecular sieves (500 mg) in dry CH_2Cl_2 (20 mL) were cooled at −20 °C. The glycosylation donor **3** (706 mg, 1.8 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise under an argon atmosphere. The mixture was stirred for 15 min, and NIS (540 mg, 2.4 mmol), followed by $Sn(OTf)_2$ (250 mg, 0.6 mmol), were added to initiate coupling. The reaction mixture was stirred for 30 min at rt, quenched by addition of Et_3N (1 mL), diluted with CH_2Cl_2 (50 mL), and filtered through a Celite pad. The filtrate was washed with 10% $Na_2S_2O_3$ (20 mL), followed by washing with satd aq $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by column chromatography (2:1 cyclohexane–AcOEt) to give disaccharide **14** as a colorless solid (718 mg, 67%): mp 74–76 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06–8.02 (m, 2H, Ar), 7.97–7.91 (m, each 4H, Ar), 7.56–7.45 (m, 3H, Ar), 7.44–7.31 (m, 6H, Ar), 5.91 (dd, 1H, $J_{3,4}$ 10.0 Hz, $J_{4,5}$ 9.7 Hz, H-4), 5.81 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.46–5.42 (m, 2H, H-2′, H-3′), 5.24 (m, 1H, H-4′), 5.13 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.96 (d, 1H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.61 (dd, 1H, $J_{5,6a}$ 3.1 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.51 (dd, 1H, $J_{5,6a}$ 5.4 Hz, $J_{6a,6b}$ 12.1 Hz, H-6b), 4.38 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6a}$ 3.1 Hz, $J_{5,6b}$ 5.4 Hz, H-5), 4.31 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.7 Hz, H-2), 4.23 (dd, 1H, $J_{5',6'a}$ 5.2 Hz, $J_{6'a,6'b}$ 11.6 Hz, H-6′a), 4.15 (ddd, 1H, $J_{4',5'}$ 9.9 Hz, $J_{5',6'a}$ 5.2 Hz, $J_{5',6'b}$ 2.3 Hz, H-5′), 4.08 (dd, 1H, $J_{5',6'b}$ 2.3 Hz, $J_{6'a,6'b}$ 12.2 Hz, H-6′b), 4.02–3.96 (m, 1H, OCH_2), 3.77–3.70 (m, 1H, OCH_2), 3.53–3.50 (m, 2H, CH_2N_3), 2.08, 2.04, 2.02, 1.99 (s, each 3H, OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.36, 169.64, 169.33, 169.27 (C=O), 166.08, 165.34, 165.05 (C=O), 133.31, 133.20, 133.89 (CH), 129.78, 129.72, 129.56 (CH), 128.84, 128.69 (C), 128.42, 128.34, 128.24 (CH), 99.31 (C-1′), 98.52 (C-1), 76.35 (C-2), 70.49 (C-3), 69.17, 69.03, 68.99 (C-5, C-2′, C-3′, C-4′), 68.65 (C-4′), 66.99 (OCH_2), 67.03 (C-4), 66.16 (C-5′), 63.42 (C-6′), 62.51 (C-6), 50.26 (CH_2N_3), 20.57, 20.54 (OCH_3). ESIMS: m/z 914.2607 $(M + Na)^+$, calcd for $C_{43}H_{45}N_3O_{18}$ 914.2590 $(M + Na)^+$.

3.1.11. 5-Azidonaphthalene-1-sulfonamidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl-(1 → 2)-3,4,6- tri-*O*-benzoyl-α-D-mannopyranoside (15). Compound **14** (687 mg, 0.77 mmol) was dissolved in dry MeOH (20 mL), and 5% Pd/C was added (103 mg) under an argon atmosphere. HCO_2NH_4 (194 mg, 3.08 mmol) was added, and the reaction mixture was stirred at rt for 45 min, filtered through a short Celite pad, and concentrated. The syrup was dissolved in $CHCl_3$ (20 mL), washed with water (2 × 10 mL), dried over Na_2SO_4, and concentrated to an oil. To a dry CH_2Cl_2 (20 mL) solution of this crude reduced disaccharide (682 mg, ~2.25 mmol) was added *N*-methylimidazole (360 μL, 4.5 mmol), and the solution was cooled to 0 °C. To this cold solution was added 5-azidonaphthalene-1-sulfonyl chloride (906 mg, 3.38 mmol) under near darkness. The reaction mixture was stirred for 12 h at 0 °C and quenched with water (10 mL). The organic layer was washed with water (2 × 10 mL), dried over Na_2SO_4, and concentrated. The pure disaccharide **15** was obtained after

column chromatography (2:1 cyclohexane–AcOEt) as a light-sensitive solid (513 mg, 61%). It was stored in a cool, dark dry place until further reaction. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.47 (d, 1H, J 8.8 Hz, Ar), 8.39 (d, 1H, J 8.5 Hz, Ar), 8.33 (dd, 1H, J 1.1, 7.4 Hz, Ar), 7.97–7.96 (m, 4H, Ar), 7.93–7.91 (m, 2H, Ar), 7.68 (dd, 1H, J 7.6, 8.8 Hz, Ar), 7.56 (dd, 1H, J 7.4, 8.5 Hz, Ar), 7.53–7.48 (m, 3H, Ar), 7.40–7.33 (m, 6H, Ar), 7.30 (d, 1H, J 7.6 Hz, Ar), 5.80 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 10.0 Hz, H-4), 5.64 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.45 (t, 1H, J 6.2 Hz, NH), 5.41–5.39 (m, 2H, H-2′, H-3′), 5.23 (dd, 1H, $J_{3'4'} = J_{4',5'} = 9.8$ Hz, H-4′), 4.91 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.88 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1′), 4.47 (dd, 1H, $J_{5',6'a}$ 3.2 Hz, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.41 (dd, 1H, $J_{5',6'b}$ 5.5 Hz, $J_{6'a,6'b}$ 12.2 Hz, H-6′b), 4.22–4.19 (m, 2H, H-6a, H-5′), 4.09–4.06 (m, 2H, H-5, H-6b), 4.05 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.3 Hz, H-2), 3.79–3.76 (m, 1H, OCH_2), 3.51–3.47 (m, 1H, OCH_2), 3.27–3.20 (m, 2H, CH_2 NH), 2.06, 2.05, 2.02, 1.99 (s, each 3H, 4×OCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.71, 169.79, 169.47, 169.38 (C═O), 166.87, 165.53, 165.16 (C═O), 137.81 (C), 134.80 (C), 133.51, 133.33, 133.01 (CH), 130.44 (CH), 129.89, 129.81, 129.64 (CH), 129.57, 129.52, 129.15 (C), 128.94, 128.59, 128.42, 128.34 (CH), 127.19 (C), 124.37, 120.82, 114.85 (CH), 99.25 (C-1′), 98.54 (C-1), 76.17 (C-2), 70.48 (C-3), 69.14, 68.89, 68.69 (C-5, C-2′, C-3′, C-4′), 67.35 (C-4), 66.98 (OCH_2), 66.49 (C-5′), 63.52 (C-6′), 62.88 (C-6), 42.84 (CH_2NH), 20.73, 20.69, 20.64, 18.73 (OCH_3). ESIMS: m/z 1119.2855 $(M + Na)^+$, calcd for $C_{53}H_{52}N_4O_{20}S$ 1119.2787 $(M + Na)^+$.

3.1.12. 5-Azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranoside (1). To a solution of disaccharide **8** (225 mg, 0.36 mmol) in dry MeOH (2 mL) was added 7 N NH_3–MeOH (10 mL) dropwise, and the reaction mixture was stirred at room temperature for 12 h. Concentration in vacuo and flash chromatography (2:1 $CHCl_3$–MeOH) gave **1** as a light-sensitive, light-yellow oil. It was dissolved in 5 mL of deionized water (5 mL), passed through a small column of Bio-Beads™ SM-4 (20–50 mesh), and lyophilized to a white fluffy solid (90 mg, 71%) that was stored in cool dark dry place until further use. ^{1}H NMR (300 MHz, CD_3OD): δ 8.49 (d, 1H, J 8.8 Hz, Ar), 8.41 (d, 1H, J 8.5 Hz, Ar), 8.26 (dd, 1H, J 1.1, 7.3 Hz, Ar), 7.72 (dd, 1H, J 7.7, 8.8 Hz, Ar), 7.61 (dd, 1H, J 7.3, 8.5 Hz, Ar), 7.49 (dd, 1H, J 0.7, 7.7 Hz, Ar), 4.75 (d, 1H, $J_{1',2'}$ 1.7 Hz, H-1′), 4.39 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 3.82–3.77 (m, 3H), 3.71-3.37 (m, 10H), 3.23–3.17 (m, 1H, OCH_2), 3.13–3.05 (m, 2H, CH_2NH). ^{13}C NMR (75 MHz, CD_3OD): δ 138.86, 137.29 (C), 130.94 (CH), 130.48 (C), 129.16 (CH), 128.42 (C), 125.57, 122.57, 116.12 (CH), 101.72 (C-1′), 101.37 (C-1), 74.31 (C-2), 73.11, 72.59, 72.07, 71.66 (C-3, C-4, C-5, C-2′, C-3′), 68.59, 68.38 (C-4′, C-5′), 67.20, 67.17 (C-6, OCH_2), 62.86 (C-6′), 43.71 (CH_2NH). ESIMS: m/z 639.1569 $(M + Na)^+$, calcd for $C_{24}H_{32}N_4O_{13}S$ 639.1578 $(M + Na)^+$. Anal. Calcd for $C_{24}H_{32}N_4O_{13}S{\cdot}2.5H_2O$: C, 43.57; H, 5.64; N, 8.46. Found: C, 43.69; H, 5.25; N, 8.31.

3.1.13. 5-Azidonaphthalene-1-sulfonamidoethyl α-D-mannopyranosyl)-(1 → 2)-α-D-mannopyranoside (2). To a solution of disaccharide **15** (394 mg, 0.36 mmol) in dry MeOH (2 mL) was added 7 N NH_3–MeOH (10 mL) dropwise, and the reaction mixture was stirred at room temperature for 12 h. Concentration in vacuo and flash chromatography (2:1 $CHCl_3$–MeOH) gave **2** as a light-sensitive, light-yellow oil. The product was dissolved in deionized water (5 mL), passed through a small column of Bio-Beads™ SM-4 (20-50 mesh), and lyophilized to a fluffy yellow solid (180 mg, 81%) that was stored in a cool, dark, dry place until needed. ^{1}H NMR (600 MHz, CD_3OD): δ 8.50 (dd, 1H, J 8.7 Hz, Ar), 8.42 (dd, 1H, J 1.1, 8.5 Hz, Ar), 8.26 (dd, 1H, J 1.1, 7.3 Hz, Ar), 7.73 (dd, 1H, J 7.7, 8.7 Hz, Ar), 7.62 (dd, 1H, J 7.4, 8.5 Hz, Ar), 7.50 (d, 1H, J 7.4 Hz, Ar), 4.89 (d, 1H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.86 (unresolved, H-1), 3.95 (dd, 1H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.2 Hz, H-2), 3.82–3.79 (m, 1H, H-6′a), 3.73 (dd, 1H, $J_{5,6}$ 2.4 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.68–3.49 (m, 10H, H-3, H-4, H-2′, H-3′, H-4′, H-6b, H-6′b), 3.37–3.30 (unresolved m, OCH_2), 3.15–3.05 (m, 2H, CH_2NH). ^{13}C NMR (75 MHz, CD_3OD): δ 138.86 (C), 137.18 (C), 130.94 (CH), 130.50 (C), 129.13 (2×CH), 128.42 (C), 125.56, 122.63, 116.11 (3×CH), 104.10 (C-1′), 99.94 (C-1), 80.29 (C-2), 74.90, 74.59 (C-2′, C-3), 72.40 (C-4), 71.88, 71.84 (C-5, C-3′), 68.89, 68.80 (C-4′, C-5′), 67.31 (OCH_2), 63.12, 62.84 (C-6, C-6′), 43.72 (CH_2NH). ESIMS: m/z 639.1562 $(M + Na)^+$, calcd for $C_{24}H_{32}N_4O_{13}S$ 639.1578 $(M + Na)^+$. Anal. Calcd for $C_{24}H_{32}N_4O_{13}S{\cdot}2.0H_2O$: C, 44.05; H, 5.58; N, 8.56. Found: C, 44.07; H, 5.43; N, 8.19.

3.2. Mannosyltransferase assay[16]

The mycobacterial membranes from *M. smegmatics* mc^2 155 and *M. Bovis* BCG were prepared and used for compound **1** and compound **2**, respectively. Compounds **1** (SRI 20723) and **2** (SRI 20731), at a range of concentrations from 0.1 to 4.0 mM (which were stored as 100 mM ethanol stocks), were dried under a stream of argon in a microcentrifuge tube (1.5 mL). The materials were placed in a vacuum desiccator for 15 min to remove any residual solvent and were then resuspended in 8 μL of a 1% aqueous solution of Igepal. The remaining components of the mannosyltransferase assay were added, consisting of 50 mM MOPS (adjusted to pH 8.0 with KOH), 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM ATP, membranes (250 μg), and GDP-[U-^{14}C]mannose (0.25 μCi, 11 GBq/mmol, Amersham) were added to a final reaction volume of 80 μL. The reaction mixtures were then incubated at 37 °C for 1 h. A $CHCl_3$–CH_3OH (1:1, 533 μL) solution was then added to the

incubation tubes, and the entire contents centrifuged at 18,000×*g*. The supernatant was recovered and dried under a stream of argon and re-suspended in 1:1 C_2H_5OH–H_2O (1 mL) and loaded onto a pre-equilibrated (1:1 C_2H_5OH–H_2O) 1-mL Whatmann strong anion-exchange (SAX) cartridge that was washed with 3 mL of ethanol. The eluate was dried, and the resulting products partitioned between the two phases arising from a mixture of *n*-butanol (3 mL) and H_2O (3 mL). The resulting organic phase was recovered following centrifugation at 3500×*g*, and the aqueous phase was again extracted twice with 3 mL of *n*-butanol-satd water. The pooled extracts were back-washed twice with water satd with *n*-butanol (3 mL). The *n*-butanol-satd water fraction was dried and re-suspended in 200 μL of *n*-butanol. The total cpm of radiolabeled material extractable into the *n*-butanol phase was measured by scintillation counting using 10% of the labeled material and 10 mL of EcoScintA (National Diagnostics, Atlanta). The incorporation of [^{14}C]Man*p* 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 TLC in 65:25:0.5:3.6 $CHCl_3$–CH_3OH–NH_4OH–H_2O on aluminum-backed Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany). Autoradiograms were obtained by exposing TLCs to X-ray film (Kodak X-Omat) for 3 days.

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