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The Galactosamine Residue in Mycobacterial Arabinogalactan Is α-Linked

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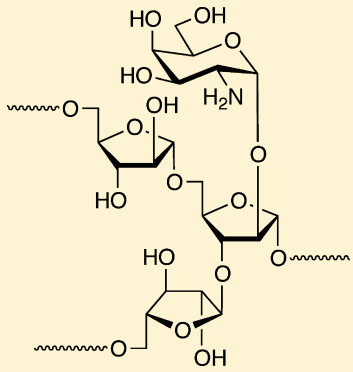
Supporting Information

ABSTRACT: Previous studies have demonstrated that cell wall arabinogalactan from mycobacteria possesses a single galactosamine (GalN) residue. This moiety, which is one of the rare natural occurrences of galactosamine lacking an acetyl group on the nitrogen, has been identified as a pendant substituent attached to a highly branched arabinofuranose residue in the arabinan core. However, the stereochemistry by which the GalN residue is linked to the polysaccharide remains unknown. We report here the synthesis of two tetrasaccharides, **1** and **2**, consisting of GalN attached through either an α- or β-linkage to a trisaccharide fragment of mycobacterial arabinan. These molecules represent the first synthetic GalN-containing oligosaccharides, and the preparation of both targets was achieved from a single donor species by modulation of the reaction solvent. Comparison of the NMR spectra of **1** and **2** with those obtained from a sample derived from the natural glycan revealed that the GalN residue in the polysaccharide is attached via an α-linkage.



Tuberculosis remains a public health concern, with estimates that a third of the world's population harbors *Mycobacterium tuberculosis*, the organism that causes the disease.[1] Although only a few of those infected will develop active disease, fears about even more widespread dissemination of tuberculosis have been heightened in recent years by the emergence of *M. tuberculosis* strains with varying degrees of drug resistance.[2] These public health issues have led to increased interest in understanding mycobacterial physiology and the organism's cell wall, a complex and intricate structure that is essential to its survival, has received particular attention.[3,4]

The major structural component of the mycobacterial cell wall is a glycolipid, the mycolyl−arabinogalactan (MAG) complex, which is covalently bound to peptidoglycan.[3] The predominant components of the MAG complex are the monosaccharides galactose and arabinose, both in their furanose ring form, and mycolic acids, which are C_{70}−C_{90} branched chain lipids characteristic to mycobacteria and related organisms (e.g., corynebacteria, nocardia). Also present is a single rhamnose and a single *N*-acetylglucosamine phosphate residue, which together form the linker that connects the MAG to the peptidoglycan. More recently, two additional components, succinic acid and 2-amino-2-deoxy-galactose (GalN), have been identified in the MAG of some mycobacterial strains, both of which are present at substoichiometric levels.[5,6]

The presence of GalN in some mycobacterial strains was proposed more than 40 years ago.[7] However, its presence was only unequivocally established more recently due to improvements in polysaccharide degradation methods and mass spectrometry.[5,6] These studies revealed that the GalN moiety (as well as the succinate modification) are attached to the arabinan domain of the MAG through O-2 on arabinofuranose (Ara*f*) residues that are further branched at both O-3 and O-5 with additional Ara*f* motifs (Figure 1A). Although glycans containing 2-acetamido-2-deoxy-galactose (GalNAc) are widespread in nature, GalN, which lacks the acetyl group, is rare. To date, this monosaccharide has been identified as a component of the lipopolysaccharide in *Francisella tularensis*,[8,9] *Legionella hackeliae*,[10] *Campylobacter jejuni*,[11] *Campylobacter coli*,[11] as well as a glycoprotein from *Bacillus thuringiensis*.[12]

The biosynthesis of GalN in mycobacteria has been studied and a lipid-linker GalNAc-pyrophosphate species was isolated and characterized.[13] In addition, the genes encoding for the synthase and transferase that assemble and use this glycosylating agent were identified. Subsequent deacetylation (either before or after glycosylation) by a to date unidentified amidase was proposed to complete the biosynthetic pathway. On the basis of similarities with an intermediate isolated from *F. tularensis*[14,15] and the sequence of the transferase, which predicts it to be an inverting enzyme, the stereochemistry of the GalN moiety was proposed to be α. However, unequivocal evidence is not available to support this stereochemical assignment. To address this issue, we report here the synthesis of both the α- and β-GalN stereoisomers of this tetrasaccharide motif (**1** and **2**, Figure 1B) and the establishment of the linkage stereochemistry by comparison of the NMR data for these compounds with that for a fragment of the natural polysaccharide.

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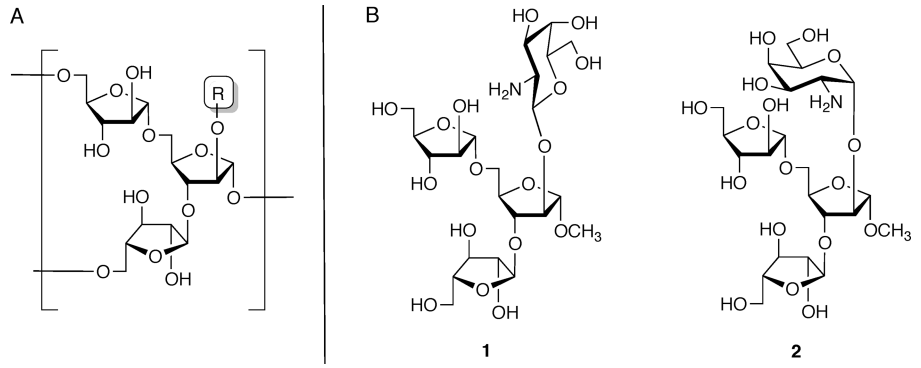
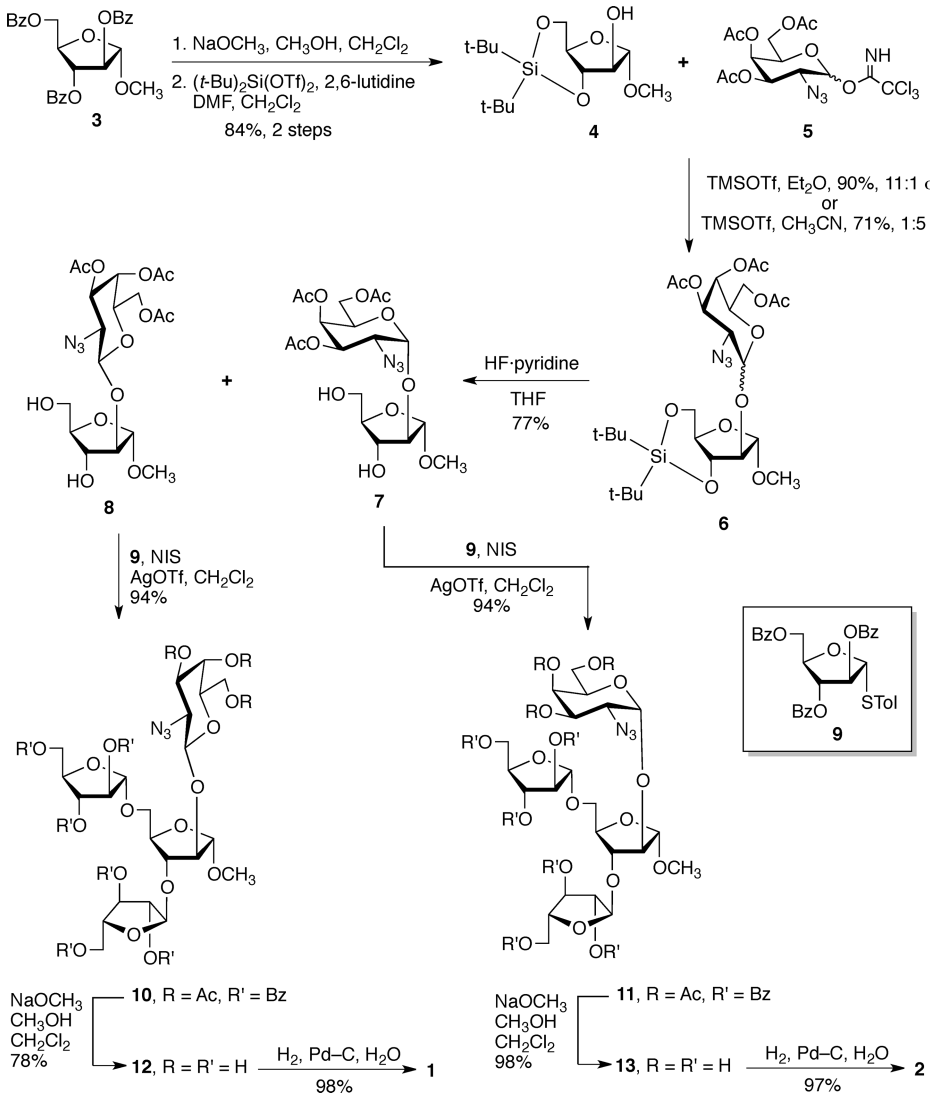


Figure 1. (A) Proposed location of succinate and GalN residues in mycobacterial arabinogalactan. (B) Tetrasaccharide targets **1** and **2**.

Scheme 1. Synthesis of 1 and 2



To the best of our knowledge, there are no previous syntheses of oligosaccharides containing GalN residues, which is perhaps not surprising given the rarity of this structural motif in nature. In addition, there are no previous syntheses of oligofuranosides containing a residue in which every hydroxyl group is glycosylated. We anticipated the synthesis of **1** and **2** could prove challenging given steric crowding that could prevent efficient reaction between hydroxyl groups and activated glycosyl donors as the substitution pattern on the core Ara*f* ring increases. Because we wanted to synthesize both GalN anomers attached to the triarabinofuranoside motif (**1** and **2**), we adopted a route involving the use of a 2-azido-2-deoxy-galactopyranosyl donor combined with modification of the reaction conditions to provide increased amounts of either the α- or β-glycoside.

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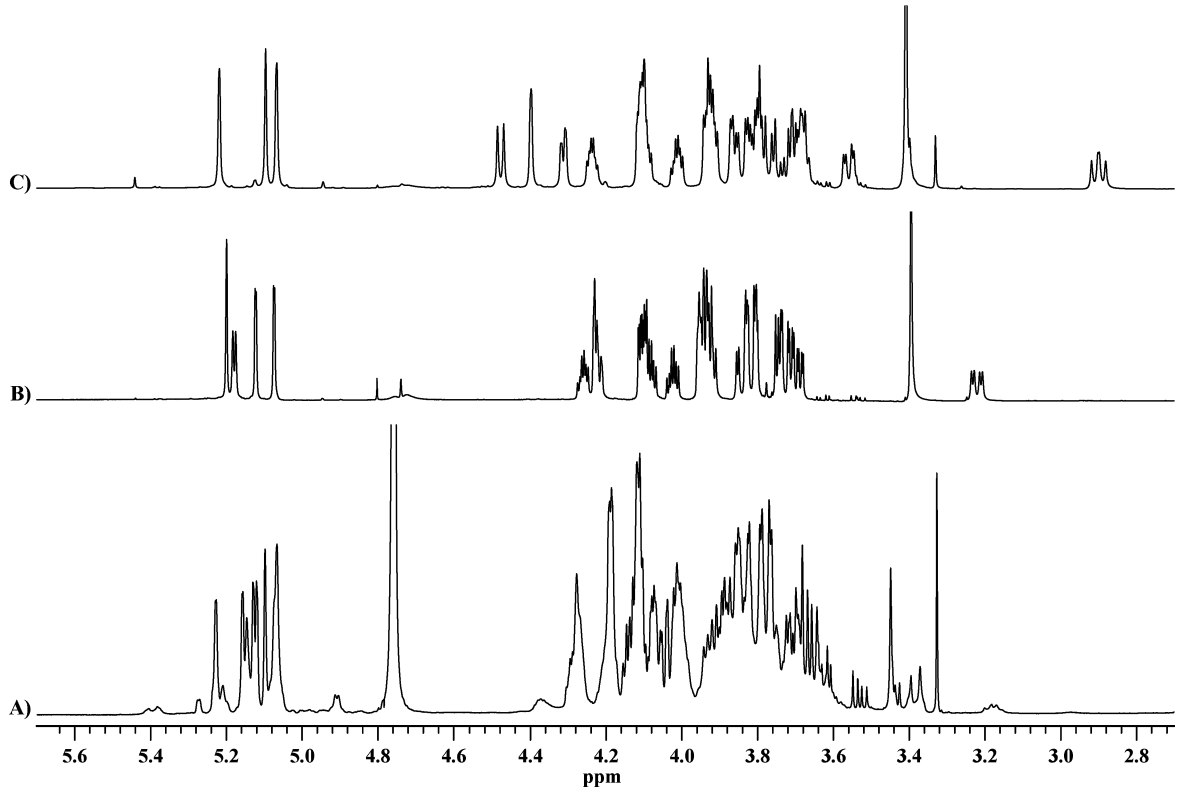

Figure 2. Comparison of the ^{1}H NMR spectrum of AG containing a GalN moiety (A) with the ^{1}H NMR spectra of **1** (C) and **2** (B).

The synthesis of **1** and **2** is illustrated in Scheme 1 and began from the methyl glycoside **3**. This monosaccharide, prepared as previously reported,[16] was converted into the silyl acetal protected derivative **4** in two steps first by debenzoylation and then treatment with di-*t*-butylsilyl bis(trifluoromethanesulfonate). The product was obtained in 84% yield over the two steps.

Subsequent glycosylation of this alcohol using trichloroacetimidate **5**[17] was carried out employing activation with trimethylsilyl trifluoromethanesulfonate using two different solvents to influence product stereochemistry. In one case, the reaction was performed using diethyl ether as the solvent,[18] which led to the product **6** in 90% yield as an inseparable 11:1 α:β mixture of isomers. In the other case, the reaction was carried out in acetonitrile,[19] resulting in a 71% yield of the product **6** as a 1:5 α:β mixture of glycosides. Thus, by using well-established solvent effects, it was possible to obtain mixtures of **6** enriched in each of the two stereoisomers.

Although separation was not possible at this stage, each mixture was carried through to the next step, treatment with HF·pyridine to remove the silyl acetal. This reaction led to the formation of **7** and **8**, which could be separated by chromatography, in a combined 77% yield.[20] Determining the stereochemistry of the pyranose residue in **7** and **8** could be done unequivocally using ^{1}H NMR spectroscopy. The anomeric hydrogen in the pyranose residue in **7** appeared as a doublet with J = 3.5 Hz, clearly supporting the α-stereochemistry. In **8**, the resonance for this hydrogen appears as a doublet with J = 8.0 Hz, consistent with the β-stereochemistry. The diol moiety in both **7** and **8** could then be glycosylated independently using an excess of known thioglycoside **9**,[21] promoted by *N*-iodosuccinimide and silver triflate[22] to afford tetrasaccharides **10** and **11**, both in 94% yield. All of the ^{1}H NMR resonances for the Ara*f* linkages in **10** and **11** appeared as singlets or as small doublets (J < 2 Hz) consistent with the α-stereochemistry.[23] Contrary to our expectation, the synthesis of the fully substituted central Ara*f* ring in **1** and **2** proved straightforward. We attribute this to the enhanced flexibility of the five-membered ring, which possesses sufficient mobility to allow efficient glycosylation of all hydroxyl groups.

Deprotection of **10** and **11** proceeded in two steps. First, each was deacylated with sodium methoxide to provide the azido-tetrasaccharides **12** and **13**, in 78 and 98% yields, respectively. The azido groups in these materials were reduced to the corresponding amines upon reaction of an aqueous solution of **12** or **13** with hydrogen and palladium on carbon. The final target compounds **1** and **2** were obtained in >97% yield in both cases.

With both tetrasaccharide targets in hand, a comparison of their NMR spectra with those obtained from the natural glycans[6] was carried out. The ^{1}H NMR spectrum of the natural glycan was expectedly complex (Figure 2A), but nevertheless it was possible to identify characteristic resonances. In particular, we focused our attention on a signal at 3.18 ppm, which appears at a chemical shift anticipated for a hydrogen on a carbon bearing a primary amine, and which integrated ∼1:1 with a signal at 4.92 ppm. The latter resonance has been unequivocally assigned previously[6] to arise from a proton on a secondary ring carbon bearing a succinate moiety, which was present in a 1:1 ratio with the GalN moiety. In addition, previous studies on intact AG indicate that none of the resonances for the other monosaccharides (arabinofuranose and galactofuranose) appear below 3.5 ppm.[24] Taking this data into consideration, we conclude that the signal at 3.18 ppm arises from the hydrogen on H-2 in the GalN ring in the

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polysaccharide. This signal matched well with the signal for H-2′ in the α-GalN-containing tetrasaccharide **2** (3.21 ppm, Figure 2B). In contrast, the H-2′ resonance in the spectrum for in β-GalN tetrasaccharide **1** was found at significantly lower chemical shift (2.90 ppm, Figure 2C). It proved impossible to identify the anomeric hydrogen on the GalN residue in the polysaccharide due to spectral overlap. However, the resonances present in the ^{1}H NMR spectra of tetrasaccharide **1** (Figure 2C) provided further evidence that the naturally occurring GalN residue is α-linked. The H-1 resonance for the β-GalN residue in **1** is found at 4.48 ppm, a region that is completely lacking in signals in the polysaccharide spectrum. Finally, further evidence came from the signal for C-2′ in the polysaccharide, 49.9 ppm,[6] obtained from an HSQC experiment (see Supporting Information). This chemical shift corresponds better with this signal in tetrasaccharide **2** (51.4 ppm) than in **1** (52.9 ppm). Taken together, these data allow us to conclude that the GalN residue in the natural glycan is α-linked. It should be noted that this is consistent with previous studies on the biosynthesis of this moiety,[13] which have suggested that the biochemical precursor is a β-linked phospholipid sugar donor and that the enzyme that adds this moiety to the arabinan is an inverting glycosyltransferase.

In conclusion, we describe here the first synthesis of GalN-containing oligosaccharides related to those found in nature. In particular, we have synthesized, for the first time, a highly branched fragment of mycobacterial AG containing the GalN substituent, consisting of an α-Araf motif glycosylated at every hydroxyl group with either GalN (O-2) or α-Araf (O-3 and O5). The key glycosylation reaction involved the reaction of a 2-azido-sugar glycosyl trichloroacetimidate; judicious choice of the solvent enabled the preparation of products enriched in either the α or β-anomer. Comparison of the NMR spectra of these compounds with those obtained from a fragment of the naturally occurring polysaccharide established that the GalN residue in the natural glycan is linked to the arabinan via an α-linkage. Now that the stereochemistry of the GalN linkage has been established, future syntheses of this motif should be facilitated by recently developed methodology for the preparation of 1,2-*cis*-2-amino-glycosides.[25−33]

EXPERIMENTAL SECTION

General Experimental Methods. All reagents were purchased from commercial sources and were used without further purification unless noted. Solvents used in reactions were purified by successive passage through columns of alumina and copper under argon. Unless stated otherwise, all reactions were carried out at room temperature and under a positive pressure of argon and monitored by TLC on Silica Gel G-25 F_{254} (0.25 mm). TLC spots were detected under UV light and/or by charring with a solution of anisaldehyde in ethanol, acetic acid and H_2SO_4. Column chromatography was performed on silica gel 60 (40−60 μm). Iatrobeads refers to a beaded silica gel 6RS−8060, which is manufactured by Iatron Laboratories (Tokyo). Organic solutions were dried using anhydrous Na_2SO_4, and solvents were evaporated under reduced pressure and below 50 °C (water bath) on a rotary evaporator. ^{1}H NMR were recorded at 400, 500, or 600 MHz, and ^{13}C NMR spectra were recorded at 100 or 125 MHz. ^{1}H NMR chemical shifts are referenced to $CHCl_3$ (7.26, $CDCl_3$) or H_2O (4.79, D_2O). ^{13}C NMR chemical shifts are referenced to $CDCl_3$ (77.0, $CDCl_3$) or external acetone (31.07, D_2O). ^{1}H NMR data are reported as though they were first order, and the peak assignments were made on the basis of 2D-NMR (^{1}H−^{1}H COSY and HSQC) experiments. Before recording the NMR spectra of **1** and **2**, the pH of a D_2O solution of each compound was adjusted to pH 7.6 by the addition of a solution of DCl in D_2O. ESI-MS spectra (time-of-flight analyzer) were recorded on samples suspended in THF or CH_3OH and with added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of deg·mL (dm·g)$^{-1}$.

Methyl 2-*O*-(2-amino-2-deoxy-β-D-galactopyranosyl)-3,5-di-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside (1). Tetrasaccharide **12** (57 mg, 0.09 mmol) was dissolved in H_2O (5 mL), and hydrogenolyzed over 10% Pd−C (15 mg) under 1 atm H_2 at room temperature for 2 h. The mixture was filtered through Celite, concentrated. The residue was dissolved in CH_3OH and passed through a polytetrafluoroethene (PTFE) syringe filter (diameter 25 mm, pore size 0.2 mm). After concentration, lyophilization of the compound from water afforded **1** (49 mg, 98%): $[\alpha]_D$ +109.6 (c = 0.8, CH_3OH); IR (film) 3353, 2929, 1082, 1047 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ = 5.22 (br. s, 1 H, H-1″), 5.10 (s, 1 H, H-1), 5.07 (br. s, 1 H, H-1‴), 4.48 (d, J = 8.0 Hz, 1 H, H-1′), 4.40 (br. s, 1 H, H-2), 4.31 (dd, J = 1.5, 5.5 Hz, 1 H, H-3), 4.25−4.22 (m, 1 H, H-4), 4.12−4.08 (m, 3 H, H-2″, H-2‴, H-4‴), 4.03−4.00 (m, 1 H, H-4″), 3.94−3.91 (m, 3 H, H-3″, H-3‴, H-5$_a$), 3.87 (d, J = 3.0 Hz, 1 H, H-4′), 3.86−3.66 (m, 8 H, H-5$_b$, H-5$_a$″, H-5$_a$‴, H-6$_a$′, H-6$_b$′, H-5$_b$‴, H-5$_b$″, H-5′), 3.56 (dd, J = 3.3, 10.5 Hz, 1 H, H-3′), 3.41 (s, 3 H, OCH_3), 2.90 (dd, J = 8.0, 10.3 Hz, 1 H, H-2′); ^{13}C NMR (125 MHz, D_2O) δ = 107.4 (C-1‴), 107.0(4), 107.0(3) (C-1, C-1″), 103.1 (C-1′), 86.0 (C-2), 84.1 (C-4″), 83.9 (C-4‴), 81.5 (C-4), 81.2, 81.1, 81.0 (C-2″, C-2‴, C-3), 76.7 (C-3″, C-3‴), 75.5 (C-5′), 72.3 (C-3′), 67.8 (C-4′), 66.2 (C-5), 61.2(0), 61.1(5), 61.0(6) (C-5″, C-5‴, C-6′), 54.7 (OCH_3), 52.9 (C-2′); HRESI-MS m/z [M + H$^+$] calcd for $C_{22}H_{40}NO_{17}$ 590.2291, found 590.2288.

Methyl 2-*O*-(2-amino-2-deoxy-α-D-galactopyranosyl)-3,5-di-*O*-(α-D-arabinofuranosyl)-α-D-arabinofuranoside (2). Compound **13** (74 mg, 0.12 mmol) was dissolved in H_2O (4 mL) and hydrogenolyzed over 10% Pd−C (20 mg) under 1 atm H_2 at room temperature for 2 h. Purification of the compound as described for **1** gave **2** (69 mg, 97%): $[\alpha]_D$ +181.5 (c = 0.7, CH_3OH); ^{1}H NMR (600 MHz, D_2O) δ = 5.20 (s, 1 H, H-1), 5.18 (d, J = 3.6 Hz, 1 H, H-1′), 5.12 (d, J = 1.2 Hz, 1 H, H-1″), 5.08 (d, J = 1.2 Hz, 1 H, H-1‴), 4.27−4.25 (m, 1 H, H-4), 4.23−4.21 (m, 2 H, H-2, H-3), 4.12−4.07 (m, 3 H, H-2″, H-2‴, H-4‴), 4.04−4.01 (m, 1 H, H-4″), 3.96−3.91 (m, 5 H, H-3″, H-3‴, H-4′, H-5′, H-5$_a$), 3.85−3.80 (m, 3 H, H-5$_b$, H-5$_a$″, H-5$_a$‴), 3.75−3.68 (m, 5H, H-6$_a$′, H-6$_b$′, H-5$_b$″, H-5$_b$‴, H-3′), 3.40 (s, 3 H, OCH_3), 3.21 (dd, J = 3.6, 10.5 Hz, 1 H, H-2′); ^{13}C NMR (150 MHz, D_2O) δ = 108.2 (C-1‴), 107.7, 107.6 (C-1″, C-1), 98.4 (C-1′), 86.1 (C-2), 85.0, 84.9(C-4″, C-4‴), 82.0(7) (C-4), 82.0(6), 81.9 (C-2″, C-2‴), 81.3 (C-3), 77.5(1), 77.4(6) (C-3″, C-3‴), 72.9 (C-4′), 69.7 (C-3′), 69.2 (C-5′), 66.9 (C-5), 62.0(9), 62.0(7), 62.0(1) (C-5″, C-5‴, C-6′), 55.5 (OCH_3), 51.4 (C-2′); HRESI-MS m/z [M + Na$^+$] calcd for $C_{22}H_{39}NO_{17}Na$ 612.2110, found 612.2114.

Methyl 3,5-*O*-di-*t*-butylsilylene-α-D-arabinofuranoside (4). To a solution of methyl glycoside **3**[16] (1.49 g, 3.13 mmol) in a mixture of CH_2Cl_2 (3 mL) and CH_3OH (20 mL) was added a catalytic amount of $NaOCH_3$ (0.1 mL 10% in CH_3OH). The reaction was stirred for 4 h at room temperature and then neutralized by the addition of Amberlite IR-120 (H$^+$) resin. The resin was removed by filtration through Celite, and the filtrate was concentrated. The resulting residue was then dissolved in a mixture of CH_2Cl_2 (5 mL) and DMF (22 mL) at 0 °C before 2,6-lutidine (1.45 mL, 4 equiv) and di-*t*-butylsilyl bis(trifluoromethanesulfonate) (1.01 mL, 1.0 equiv) were added. The mixture was stirred for 4 h at room temperature before being diluted with CH_2Cl_2. The organic solution was washed successively with water and brine and then dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel (Hexane−EtOAc, 7:1 → 6:1) to afford **4** (800 mg, 84% over two steps) as a white solid: R_f = 0.39 (Hexane−EtOAc, 4:1); $[\alpha]_D$ +57.7 (c = 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.81 (d, J = 3.6 Hz, 1 H, H-1), 4.30−4.38 (m, 1 H, H-5$_a$), 4.12 (dd, J = 3.6, 7.2 Hz, 1 H, H-2), 4.00−3.89 (m, 3 H, H-3, H-4, H-5$_b$), 3.42 (s, 3 H, OCH_3), 1.06 (s, 9 H, C(CH_3)$_3$), 1.00 (s, 3 H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 109.0 (C-1), 81.6 (C-2, C-3), 73.7 (C-4), 67.5 (C-5), 56.1 (OCH_3), 27.4 (C(CH_3)$_3$), 27.1 (C(CH_3)$_3$), 22.6 (C(CH_3)$_3$), 20.1 (C(CH_3)$_3$); HRESI-MS m/z [M + Na$^+$] calcd for $C_{14}H_{28}O_5SiNa$ 327.1598, found 327.1598.

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Methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α/β-D-galactopyranosyl-(1→2)-3,5-*O*-di-*t*-butylsilylene-α-D-arabinofuranoside (6). *Method A.* A mixture of alcohol **4** (120 mg, 0.39 mmol), imidate **5**[17] (282 mg, 1.5 equiv), and powdered 4 Å molecular sieves in anhydrous Et_2O (6 mL) was stirred at −20 °C under Ar for 30 min. A solution of TMSOTf in Et_2O (0.1 equiv) was added dropwise, the solution was stirred at −20 °C for 2 h, and then Et_3N was added. After filtration of the mixture through Celite and concentration of the filtrate, the residue was purified by chromatography (Hexane−EtOAc, 5:1) to give disaccharide **6** as an inseparable 11:1 α:β mixture (217 mg, 90%) as a white solid: R_f = 0.28 (Hexane−EtOAc, 4:1). A small amount of unreacted acceptor **4** (4 mg, 3%) was also recovered. Data for α-isomer: ^{1}H NMR (600 MHz, $CDCl_3$) δ = 5.46 (dd, *J* = 1.2, 3.0 Hz, 1 H, H-4′), 5.41 (d, *J* = 3.0 Hz, 1 H, H-1′), 5.35 (dd, *J* = 3.0, 11.4 Hz, 1 H, H-3′), 4.87 (d, *J* = 3.6 Hz, 1 H, H-1), 4.30−4.38 (m, 1 H, H-5_a), 4.24−4.28 (m, 1 H, H-5′), 4.20−4.11 (m, 3 H, H-6_a′, H-2, H-3), 4.06 (dd, *J* = 7.2, 11.4 Hz, 1 H, H-6_b′), 3.96−3.92 (m, 2 H, H-4, H-5_b), 3.69 (dd, *J* = 3.0, 11.4 Hz, 1 H, H-2′), 3.42 (s, 3 H, OCH_3), 2.14 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 1.06 (s, 9 H, $C(CH_3)_3$), 0.99 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.0 (C═O), 169.9 (C═O), 106.9 (C-1), 97.4 (C-1′), 85.8 (C-2), 81.0 (C-3), 73.6 (C-4), 67.9 (C-3′), 67.5 (C-4′), 67.4 (C-5), 66.9 (C-5′), 61.5 (C-6′), 57.3 (C-2′), 56.1 (OCH_3), 27.3(6) ($C(CH_3)_3$), 27.3(1) ($C(CH_3)_3$), 27.0 ($C(CH_3)_3$), 22.6 ($C(CH_3)_3$), 20.6(5) (CH_3CO), 20.6(3) (CH_3CO), 20.5(9) (CH_3CO), 20.1 ($C(CH_3)_3$); HRESI-MS *m*/*z* [M + Na^+] calcd for $C_{26}H_{43}N_3O_{12}SiNa$ 640.2508, found 640.2507.

Method B. A mixture of alcohol **4** (95 mg, 0.31 mmol), imidate **5**[17] (243 mg, 1.6 equiv), and powdered 4 Å molecular sieves in anhydrous CH_3CN (10 mL) was stirred at −30 °C under Ar for 30 min. A solution of TMSOTf in CH_3CN (0.15 equiv) was added dropwise. After stirring at −30 °C for 4 h, Et_3N was added, and then the solution was then filtered through Celite and concentrated. Purification of the residue by chromatography (Hexane−EtOAc 5:1) gave disaccharide **6** as a 1:5 α:β ratio (136 mg, 71%) as a white solid: R_f = 0.28 (Hexane−EtOAc, 4:1). Unreacted **4** (19 mg, 20%) was also recovered. Data for β-isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.34 (dd, *J* = 1.0, 3.5 Hz, 1 H, H-4′), 4.92 (d, *J* = 3.0 Hz, 1 H, H-1), 4.82 (dd, *J* = 3.5, 11.0 Hz, 1 H, H-3′), 4.53 (d, *J* = 8.0 Hz, 1 H, H-1′), 4.30−4.38 (m, 1 H, H-5_a), 4.22−4.10 (m, 4 H, H-2, H-3, H-6_a′, H-6_b′), 3.97−3.92 (m, 2 H, H-4, H-5_b), 3.88 (dt, *J* = 1.0, 7.0 Hz, 1 H, H-5′), 3.69 (dd, *J* = 8.0, 11.0 Hz, 1 H, H-2′), 3.41 (s, 3 H, OCH_3), 2.16 (s, 3 H, 3 CH_3CO), 2.06 (s, 3 H, 3 CH_3CO), 2.02 (s, 3 H, 3 CH_3CO), 1.06 (s, 9 H, $C(CH_3)_3$), 1.00 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.2, 170.1, 107.7 (C-1), 101.6 (C-1′), 88.8 (C-2), 80.2 (C-3), 74.0 (C-4), 71.0 (C-3′), 70.8 (C-5′), 67.3 (C-5), 66.0 (C-4′), 60.9 (C-2′), 60.6 (C-6′), 56.0 (OCH_3), 27.3(6) ($C(CH_3)_3$), 27.3(2) ($C(CH_3)_3$), 27.1(5) ($C(CH_3)_3$), 27.1 ($C(CH_3)_3$), 27.0 ($C(CH_3)_3$), 22.6 ($C(CH_3)_3$), 20.6(5) (CH_3CO), 20.6(3) (CH_3CO), 20.6 ($C(CH_3)_3$).

Methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl-(1→2)-α-D-arabinofuranoside (7) and Methyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl-(1→2)-α-D-arabinofuranoside (8). To a stirred solution of **6** (366 mg, 0.59 mmol, synthesized via method A) in THF (10 mL) and pyridine (2 mL) was added HF·pyridine (0.05 mL). After stirring overnight at room temperature, a saturated aq solution of $NaHCO_3$ was added, and the mixture was extracted with EtOAc. The organic layer was washed with HCl (1 N) and saturated aq $NaHCO_3$ and then dried (Na_2SO_4). The organic layer was filtered through filter funnel, and the filtrate was concentrated to give a residue that was purified by chromatography (toluene−CH_3OH, 25:1) to afford **7** (200 mg, 71%) and **8** (18 mg, 6%) both as white foams. Data for **7**: $[\alpha]_D$ +135.0 (*c* = 0.9, $CHCl_3$); IR (film) 3468, 2937, 2113, 1751, 1373, 1231, 1080, 1034 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.45 (d, *J* = 3.0 Hz, 1 H, H-4′), 5.28 (dd, *J* = 3.0, 11.0 Hz, 1 H, H-3′), 5.19 (d, *J* = 3.5 Hz, 1 H, H-1′), 5.03 (s, 1 H, H-1), 4.15−4.40 (m, 1 H, H-5′), 4.14 (dd, *J* = 6.0, 11.5 Hz, 1 H, H-6_a′), 4.13−4.03 (m, 4 H, H-2, H-3, H-4, H-6_b′), 3.84 (dd, *J* = 4.0, 12.0 Hz, 1 H, H-5_a), 3.78−3.72 (m, 2 H, H-2′, H-5_b), 3.42 (s, 3 H, OCH_3), 2.15 (s, 3 H, 3 CH_3CO), 2.08 (s, 3 H, 3 CH_3CO), 2.06 (s, 3 H, 3 CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.7 (C═O), 170.0 (C═O), 106.5 (C-1), 97.5 (C-1′), 87.1 (C-2), 85.0 (C-3), 75.5 (C-4), 68.3 (C-3′), 67.4 (C-4′), 67.3 (C-5′), 62.3 (C-5), 61.9 (C-6′), 57.4 (C-2′), 55.0 (OCH_3), 20.7 (CH_3CO), 20.6(2) (CH_3CO), 20.5(8) (CH_3CO); HRESI-MS *m*/*z* [M + Na^+] calcd for $C_{18}H_{27}N_3O_{12}Na$ 500.1487, found 500.1486. Data for **8**: $[\alpha]_D$ +49.0 (*c* = 0.7, $CHCl_3$); IR (film) 3532, 2933, 2116, 1751, 1370, 1237, 1081, 1039 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.30 (d, *J* = 3.0 Hz, 1 H, H-4′), 4.93 (dd, *J* = 2.0 Hz, 1 H, H-1), 4.75 (dd, *J* = 3.0, 11.0 Hz, 1 H, H-3′), 4.48 (d, *J* = 8.0 Hz, 1 H, H-1′), 4.26 (dd, *J* = 4.0, 12.5 Hz, 1 H, H-6_a′), 4.10−4.20 (m, 1 H, H-3), 4.07−3.97 (m, 3 H, H-2, H-4, H-6_b′), 3.92 (dd, *J* = 4.0, 8.5 Hz, 1 H, H-5′), 3.86 (dd, *J* = 2.5, 12.0 Hz, 1 H, H-5_a), 3.76−3.67 (m, 2 H, H-2′, H-5_b), 3.40 (s, 3 H, OCH_3), 3.35 (d, *J* = 4.0 Hz, 1 H, OH_3), 2.27 (brs, 1 H, OH_5), 2.14, 2.05, 2.02 (3 s, 9 H, 3 CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.4 (C═O), 170.0 (C═O), 169.7 (C═O), 107.0 (C-1), 102.7 (C-1′), 92.6 (C-2), 82.1 (C-4), 75.5 (C-3), 71.3 (C-5′), 70.6 (C-3′), 66.5 (C-4′), 62.3 (C-6′), 61.6 (C-5), 60.5 (C-2′), 55.6 (OCH_3), 20.5(4) (CH_3CO), 20.5(1) (CH_3CO); HRESI-MS *m*/*z* [M + Na^+] calcd for $C_{18}H_{27}N_3O_{12}Na$ 500.1487, found 500.1487.

Methyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-3,5-di-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (10). A suspension of diol **8** (72 mg, 0.15 mmol), thioglycoside donor **9**[21] (206 mg, 2.4 equiv), and powdered 4 Å molecular sieves in anhydrous CH_2Cl_2 (5 mL) was stirred at −20 °C under Ar for 30 min. Then NIS (85 mg, 2.5 equiv) and AgOTf (12 mg, 0.3 equiv) were added. After stirring for another 2 h at −20 °C, Et3N was added, the mixture was filtered through Celite, and the filtrate was concentrated. The resulting residue was purified by chromotography (Hexane−EtOAc, 2:1) to give **10** (192 mg, 94%) as a white solid: R_f = 0.54 (Hexane−EtOAc, 1:1); $[\alpha]_D$ +4.1 (*c* = 0.6, $CHCl_3$); IR (film) 2936, 2115, 1751, 1725, 1452, 1316, 1269, 1178, 1111, 1071, 1027, 757, 712 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.10−7.94 (m, 12 H), 7.62−7.35 (m, 14 H), 7.31−7.21 (m, 4 H), 5.59−5.55 (m, 3 H, H-3″, H-3‴, H-2‴), 5.52 (d, *J* = 1.2 Hz, 1 H, H-2″), 5.49 (s, 1 H, H-1″), 5.37 (s, 1 H, H-1‴), 5.26 (dd, *J* = 0.6, 3.0 Hz, 1 H, H-4′), 5.04 (s, 1 H, H-1), 4.82 (dd, *J* = 3.0, 12.0 Hz, 1 H, H-5a″), 4.80 (dd, *J* = 3.0, 11.4 Hz, 1 H, H-3′), 4.75 (dd, *J* = 3.6, 11.4 Hz, 1 H, H-5_a‴), 3.70−3.60 (m, 4 H, H-5_b″, H-5_b‴, H-4″, H-4‴), 4.57 (d, *J* = 8.4 Hz, 1 H, H-1′), 4.51−4.48 (m, 2 H, H-2, H-3), 4.28−4.32 (m, 1 H, H-4), 4.08−4.02 (m, 2 H, H-5_a, H-6_a′), 3.99 (dd, *J* = 6.0, 11.4 Hz, 1 H, H-6_b′), 3.92 (dd, *J* = 2.4, 11.4 Hz, 1 H, H-5_b), 3.88 (dt, *J* = 1.2, 6.0 Hz, 1 H, H-5′), 3.67 (dd, *J* = 8.4, 11.4 Hz, 1 H, H-2′), 3.38 (s, 3 H, OCH_3), 2.13, 2.02 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 1.89 (s, 3 H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 170.0 (C═O), 169.7 (C═O), 166.2 (C═O), 166.0 (C═O), 165.7 (C═O), 165.6 (C═O), 165.3 (C═O), 133.6(2) (Ar), 133.5(6) (Ar), 133.4(4) (Ar), 133.3(7) (Ar), 133.0(7) (Ar), 129.9(5) (Ar), 129.8(5) (Ar), 129.7(8) (Ar), 129.7(5) (Ar), 129.7(1) (Ar), 129.6(9) (Ar), 129.6 (Ar), 129.2, 129.1 (Ar), 129.0 (Ar), 128.8 (Ar), 128.6 (Ar), 128.5 (Ar), 128.4 (Ar), 128.3 (Ar), 128.2 (Ar), 107.2 (C-1), 105.6, 105.4 (C-1″, C-1‴), 102.1 (C-1′), 88.9 (C-2), 81.9, 81.8 (C-2″, C-3), 81.5(3), 81.4(6) (C-4, C-4″, C-4‴), 80.7 (C-2‴), 77.8, 77.5 (C-3″, C-3‴), 70.8(4), 70.8(0) (C-3′, C-5′), 66.1 (C-4′), 66.0 (C-5), 63.7, 63.6 (C-5″, C-5‴), 60.9 (C-6′), 60.7 (C-2′), 54.9 (OCH_3), 20.6 (CH_3CO), 20.4 (CH_3CO); HRESI-MS *m*/*z* [M + Na^+] calcd for $C_{70}H_{67}N_3O_{26}Na$ 1388.3905, found 1388.3906.

Methyl 2-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-3,5-di-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-α-D-arabinofuranoside (11). A suspension of diol **7** (48.6 mg, 0.1 mmol), thioglycoside donor **9**[21] (133 mg, 2.3 equiv), and powdered 4 Å molecular sieves in anhydrous CH_2Cl_2 (3 mL) was stirred at −25 °C under Ar for 30 min. Then, NIS (55 mg, 2.4 equiv) and AgOTf (8 mg, 0.3 equiv) were added, and the mixture was stirred for another 2 h at −25 °C. The addition of Et_3N was followed by filtration through Celite, and the filtrate was concentrated. The resulting residue was purified by chromatography (Hexane−EtOAc, 2:1) to give **11** (130 mg, 94%) as a white solid: R_f = 0.47 (Hexane−EtOAc, 1:1); $[\alpha]_D$ +44.9 (*c* = 0.8, $CHCl_3$); IR (film) 2938, 2112, 1724, 1269, 1111, 1071, 1028, 712 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$) δ = 8.10−7.94 (m, 12 H), 7.64−7.34 (m, 14 H), 7.31−7.22 (m, 4 H),

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5.62−5.57 (m, 3 H, H-3″, H-3‴, H-2‴), 5.53 (s, 1 H, H-2″), 5.51 (s, 1 H, H-1‴), 5.46 (d, J = 3.0 Hz, 1 H, H-4′), 5.37−5.28 (m, 3 H, H-1″, H-3′, H-1′), 5.01 (d, J = 1.2 Hz, 1 H, H-1), 4.80 (dd, J = 3.0, 12.0 Hz, 1 H, H-5_a‴), 4.72−4.76 (m, 1 H, H-5_a″), 4.68−4.62 (m, 4 H, H-4″, H-4‴, H-5_b″, H-5_b‴), 4.37 (dd, J = 3.6, 6.6 Hz, 1 H, H-3), 4.32−4.34 (m, 1 H, H-2), 4.28−4.32 (m, 1 H, H-5′), 4.24−4.27 (m, 1 H, H-4), 4.13 (dd, J = 6.0, 11.4 Hz, 1 H, H-6_a′), 4.10−4.02 (m, 2 H, H-5_a, H-6_b′), 3.90 (dd, J = 2.4, 11.4 Hz, 1 H, H-5_b), 3.73 (dd, J = 2.4, 10.8 Hz, 1 H, H-2′), 3.38 (s, 3 H, OCH_3), 2.13 (s, 3 H, 3 CH_3CO), 2.02 (s, 3 H, 3 CH_3CO), 1.96 (s, 3 H, 3 CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 170.0 (C═O), 169.5 (C═O), 166.2 (C═O), 166.0 (C═O), 165.7 (C═O), 165.6 (C═O), 165.4 (C═O), 165.3 (C═O), 133.6 (Ar), 133.4 (Ar), 133.3 (Ar), 133.0(4 (Ar), 132.9(6) (Ar), 129.9 (Ar), 129.8 (Ar), 129.7(6) (Ar), 129.7(2) (Ar), 129.6(8) (Ar), 129.6 (Ar), 129.2 (Ar), 129.1 (Ar), 129.0 (Ar), 128.9 (Ar), 128.5(4) (Ar), 128.4(9) (Ar), 128.4(6) (Ar), 128.3(5) (Ar), 128.2(9) (Ar), 128.2 (Ar), 106.3 (C-1), 105.9, 105.8 (C-1″, C-1‴), 97.8 (C-1′), 87.6 (C-2), 82.2, 81.9 (C-2″, C-2‴), 81.4, 81.3(2), 81.2(9) (C-3, C-4″, C-4‴), 79.6 (C-4), 77.7, 77.5 (C-3″, C-3‴), 68.6 (C-3′), 67.5 (C-4′), 67.2 (C-5′), 66.1 (C-5), 63.7 (C-5″, C-5‴), 61.7 (C-6′), 57.5 (C-2′), 55.1 (OCH_3), 20.6 (CH_3CO), 20.5 (CH_3CO); HRESI-MS m/z [M + Na^+] calcd for $C_{70}H_{67}N_3O_{26}Na$ 1388.3905, found 1388.3894.

Methyl 2-*O*-(2-azido-2-deoxy-*β*-D-galactopyranosyl)-3,5-di-*O*-(*α*-D-arabinofuranosyl)-*α*-D-arabinofuranoside (12). A solution of **10** (192 mg) in CH_2Cl_2 (2 mL) and CH_3OH (3 mL) was treated with catalytic amount $NaOCH_3$ solution (1 M in CH_3OH, 0.1 mL) under Ar at room temperature. After stirring overnight at room temperature, the mixture was neutralized by the addition of Amberlite IR-120 (H^+) resin. After filtration and concentration, the residue was purified by chromatography on Iatrobeads (CH_2Cl_2−CH_3OH 4:1) to give **12** (67 mg, 78%) as an oil: R_f = 0.46 (CH_2Cl_2−CH_3OH, 3:1); $[\alpha]_D$ +72.8 (c = 0.4, CH_3OH); IR (film) 3349, 2938, 2118, 1606, 1078, 1036 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ = 5.23 (d, J = 1.5 Hz, 1 H, H-1″), 5.16 (s, 1 H, H-1), 5.08 (d, J = 1.5 Hz, 1 H, H-1‴), 4.64 (d, J = 7.5 Hz, 1 H, H-1′), 4.45 (d, J = 1.0 Hz, 1 H, H-2), 4.33 (dd, J = 1.0, 5.5 Hz, 1 H, H-3), 4.24−4.30 (m, 1 H, H-4), 4.13 (dd, J = 1.5, 3.5 Hz, 1 H, H-2‴), 4.12 (dd, J = 1.5, 3.0 Hz, 1 H, H-2″), 4.08−4.11 (m, 1 H, H-4‴), 4.01−4.06 (m, 1 H, H-4″), 3.97−3.90 (m, 4 H, H-3″, H-3‴, H-4′, H-5_a), 3.87−3.64 (m, 8 H, H-5_b, H-5_a″, H-5_a‴, H-6_a′, H-6_b′, H-5_b″, H-5_b‴, H-5′), 3.61 (dd, J = 3.5, 10.5 Hz, 1 H, H-3′), 3.54 (dd, J = 7.5, 10.5 Hz, 1 H, H-2′), 3.43 (s, 3 H, OCH_3); ^{13}C NMR (125 MHz, D_2O) δ = 108.3 (C-1‴), 108.0 (C-1″), 107.9 (C-1), 102.7 (C-1′), 87.0 (C-2), 84.9, 84.7 (C-4″, C-4‴), 82.5 (C-4), 82.0 (C-2″, C-2‴, C-3), 77.6, 77.5 (C-3″, C-3‴), 76.2 (C-5′), 72.1 (C-3′), 69.7 (C-4′), 67.2 (C-5), 64.2 (C-2′), 62.0(4), 62.0, 61.7 (C-5″, C-5‴, C-6′), 55.6 (OCH_3); HRESI-MS m/z [M + Na^+] calcd for $C_{22}H_{37}N_3O_{17}Na$ 638.2015, found 638.2019.

Methyl 2-*O*-(2-azido-2-deoxy-*α*-D-galactopyranosyl)-3,5-di-*O*-(*α*-D-arabinofuranosyl)-*α*-D-arabinofuranoside (13). A solution of **11** (178 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) and CH_3OH (10 mL) was treated with a catalytic amount of $NaOCH_3$ solution (1 M in CH_3OH, 0.1 mL). After stirring overnight at room temperature, the solution was neutralized by the addition of Amberlite IR-120 (H^+) resin. After filteration through Celite and concentration of the filtrate, the resulting residue was purified by chromatography on Iatrobeads (CH_2Cl_2−CH_3OH, 4:1) to give **13** (77 mg, 96%) as an oil: R_f = 0.48 (CH_2Cl_2−CH_3OH, 3:1); $[\alpha]_D$ +186.8 (c = 0.9, CH_3OH); IR (film) 3380, 2931, 2112, 1643, 1451, 1413, 1318, 1230, 1038 cm^{-1}; ^{1}H NMR (600 MHz, D_2O) δ = 5.27 (d, J = 3.0 Hz, 1 H, H-1′), 5.19 (s, 1 H, H-1), 5.18 (d, J = 0.6 Hz, 1 H, H-1″), 5.09 (d, J = 1.8 Hz, 1 H, H-1‴), 4.30−4.25 (m, 3 H, H-4, H-3, H-2), 4.15−4.13 (m, 2 H, H-2″, H-2‴), 4.09−4.11 (m, 1 H, H-4‴), 4.08−4.03 (m, 3 H, H-3′, H-4′, H-4″), 3.98−3.94 (m, 4 H, H-3″, H-3‴, H-5′, H-5_a), 3.88−3.82 (m, 3 H, H-5_b, H-5_a″, H-5_a‴), 3.78−3.71 (m, 4 H, H-5_b″, H-5_b‴, H-6_a′, H-6_b′), 3.61 (dd, J = 3.6, 10.8 Hz, 1 H, H-2′), 3.42 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, D_2O) δ = 108.2 (C-1‴), 108.0 (C-1″), 107.6 (C-1), 98.6 (C-1′), 86.3 (C-2), 84.9 (C-4″), 84.6 (C-4‴), 82.1, 82.0 (C-4, C-2″, C-2‴), 81.4 (C-3), 77.5, 77.4 (C-3″, C-3‴), 72.7 (C-5′), 69.7 (C-4′), 68.4 (C-3′), 67.1 (C-5), 62.0, 61.9(8), 61.9(4) (C-5″, C-5‴, C-6′), 60.4 (C-2′), 55.6 (OCH_3); HRESI-MS m/z [M + Na^+] calcd for $C_{22}H_{37}N_3O_{17}Na$ 638.2015, found 638.2018.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra of new compounds and the HSQC spectrum of the polysaccharide. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(20) This yield shown in Scheme 1 is that obtained starting with the mixture of **6** enriched in the α-isomer. The products could be obtained in comparable yield from the mixture of **6** enriched in the β-isomer.

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