Synthesis of a hexasaccharide that relates to the arabinogalactan epitope

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Abstract

A hexasaccharide derivative of the arabinogalactan epitope, methyl β-D-galactopyranosyl-(1 → 6)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-[α-L-arabinofuranosyl-(1 → 3)]-α-D-galactopyranosyl, was synthesized efficiently using a 3 + 3 strategy. The key step is the preparation of the trisaccharide donor, isopropyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1 → 6)-[2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1 → 3)]-2,4-di-O-benzoyl-1-thio-β-D-galactopyranoside, from isopropyl 1-thio-β-D-galactopyranoside using a one-pot synthesis of a 3,6-differentially protected building block. © 2001 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Antibodies generated against specific plant cell-wall carbohydrates may serve as probes in identifying cognate structural elements in the outer membrane saccharides of other plant species. The usefulness of this concept relies on characterization and preparation of the epitopes recognized by the corresponding antibodies. As part of our ongoing research project on the synthesis of epitopes related to arabinogalactan proteins (AGPs), we have synthesized dodecyl β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)-[α-L-arabinofuranosyl-(1 → 2)]-β-D-galactopyranoside. The synthesis of a 2-O-arabinofuranosylated β-D-(1 → 6)-linked galactan based on 1,2-anhydrosugars had been reported earlier by van Boom’s group. There has been no unambiguous structural definition on CCRC-M7-recognized arabinogalactan epitopes so far reported. The results of a series of immunoassays indicated that arabinosyl residues constitute an important part of the epitope. However, the number of arabinosyl residues in this epitope and the site of their attachment to the galactan backbone remain to be established. Besides, it is very difficult to gain arabinose-containing oligosaccharides with clear structural information for biological studies from natural resources. We present here the first synthesis of a well-defined arabinogalactan hexasaccharide derivative using our newly developed methodology for the synthesis of 3,6-branched oligosaccharides.

2. Results and discussion

Isopropyl 2,4-di-O-benzoyl-3-O-tert-butyldimethylsilyl-6-O-triphenylmethyl-1-thio-β-D-
galactopyranoside (1) was prepared from commercially available isopropyl 1-thio-β-D-galactopyranoside (IPTG) according to our previous method.\(^6\) FeCl\(_3\) hexahydrate catalyzed detritylation\(^7\) was carried out smoothly on compound 1 providing the 6-OH acceptor 2 in 85.5% yield. Standard glycosylation of 2 with fully benzoylated galactopyranosyl imidate 3 in anhydrous CH\(_2\)Cl\(_2\) gave (1 → 6)-linked di-galactopyranoside 4, followed by desilylation with 90% trifluoroacetic acid (TFA), afforded the 3-OH derivative 5 in a total yield of 64.7%. Coupling of disaccharide acceptor 5 with the arabinofuranosyl trichloroacetimidate 6 gave thioglycoside 7 as a latent trisaccharide donor in 78% yield. To synthesize the acceptor part for the target molecule assembly, building blocks 9 and 13 were each synthesized. Thus, compound 8 was selectively silylated on the primary hydroxyl group with tert-butylchlorodiphenylsilane, then benzoylated in situ with benzoyl chloride in pyridine, to afford 9 in a total yield of 74.4%. Synthon 13 was obtained by selective benzylation of 10,\(^8\) followed by removal of the acetonide group in 90% TFA (→ 12) and regioselective protection of the primary hydroxyl group with tert-butylchlorodiphenylsilane in pyridine. A doublet of doublets at δ 5.16 ppm clearly indicates the benzoylation of the 2-OH in 12 based on decoupled \(^1\)H NMR analysis. It is noteworthy that direct condensation of 11 with 6 in the presence of TMSOTf was troublesome providing significant byproducts due to the lack of stability of the 4,6-acetonide of 11. Glycosylation of diol 13 with trichloroacetimidate 6 in anhydrous CH\(_2\)Cl\(_2\) using TMSOTf (10% equiv) as catalyst gave the (1 → 3)-linked disaccharide 14 in 71.8% yield. The correct regioselectivity of 14 was confirmed by 2D \(^1\)H–\(^1\)H COSY spectroscopy. Desilylation of 14 in 90% TFA (→ 15), followed by NIS–TMSOTf catalyzed glycosylation with 9 in anhydrous CH\(_2\)Cl\(_2\) below −15°C furnished trisaccharide 16 in good yield. Acetylation of the remaining 4-OH of 16 with acetic anhydride in pyridine gave 17, which was then treated with 90% TFA to complete trisaccharide acceptor 18. Coupling reaction of trisaccharide donor 7 and trisaccharide acceptor 18 proceeded in anhydrous dichloromethane in the presence of NIS (2.5 equiv) and TMSOTf (13% equiv) to give fully protected hexasaccharide 19 (83.1%). Peaks at δ 97.9, 100.8, 100.9, 101.6, 107.5, 107.7 in the \(^{13}\)C NMR spectrum show all the C-1s in this structure. Finally, deacylation of 19 in ammonium-saturated methanol completed the synthesis of the target compound, methyl β-D-galactopyranosyl-(1 → 6)-[z-L-arabinofuranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 6)-([β-D-galactopyranosyl-(1 → 6)-[z-L-arabinofuranosyl-(1 → 3)]-z-D-galactopyranoside (20), in 97.6% isolated yield. The potential bioactivity of compound 20 is currently under investigation (Scheme 1).

3. Experimental

**General methods.**—Optical rotations were determined at 20°C with a Perkin–Elmer model 241-Mc automatic polarimeter. Melting points were determined with a ‘Mel-Temp’ apparatus. \(^1\)H, \(^{13}\)C NMR and \(^1\)H–\(^1\)H COSY spectra were recorded with ARX 400 spectrometers for solutions in CDCl\(_3\), MeOD and D\(_2\)O. Chemical shifts are given in ppm downfield from internal Me\(_4\)Si, or DSS in the case of D\(_2\)O. Mass spectra were measured using MALDI-TOF MS with α-cyano-4-hydroxycinnamic acid (CCA) as matrix or recorded with a VG PLATFORM mass spectrometer using the ESI technique to introduce the sample. Thin-layer chromatography (TLC) was performed on Silica Gel HF\(_254\) with detection by charring with 30% (v/v) H\(_2\)SO\(_4\) in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90°C) as the eluent. Solutions were concentrated at < 60°C under diminished pressure.

**Isopropyl 2,4-di-O-benzoyl-3-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside (2).**—Ferrocene hexahydrate (2.0 equiv) was added to a mixture of 1 (4.24 g, 5.29 mmol) in dry CH\(_2\)Cl\(_2\) (30 mL). The mixture was stirred at rt for 3 h, then diluted with more CH\(_2\)Cl\(_2\) (50 mL), and washed twice with ice-cold water. The washings were re-extracted with CH\(_2\)Cl\(_2\) (20 mL). The combined organic
Scheme 1.
Phase was dried and concentrated, then subjected to a silica gel column using 3:1 petroleum ether–EtOAc as eluent to give crystalline 2 (2.53 g, 85.5%); mp 110–111°C; [z]D's +76° (c 1, CHCl3); 1H NMR (CDCl3): δ 0.01 (s, 3 H, SiCH3), 0.16 (s, 3 H, SiCHOH), 0.74 (s, 9 H, C(CH3)2), 1.41 (d, 3 H, CH3), 1.44 (d, 3 H, CH3), 3.39 (m, 1 H, CH), 3.72 (q, 1 H, J6a,6b 11.1, J6a,6b 6.3 Hz, H-6a), 3.93 (q, 1 H, J6b,5 5.7 Hz, H-6b), 4.02 (t, 1 H, H-5), 4.28 (d, 1 H, J1, J3,6 1.0 Hz, H-3), 4.88 (d, 1 H, J1, J1,2 9.9 Hz, H-1), 5.62 (d, 1 H, H-4), 5.73 (t, 1 H, J1, J3,6 9.6 Hz, H-2), 7.58–8.28 (m, 10 H, PhCO). Anal. Caled for C29H40O7Si: C, 62.14; H, 7.14. Found: C, 62.05; H, 7.29.

Isopropyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-O-benzoyl-3-O-tet-butylidemethylsilyl-1-thio-β-D-galactopyranoside (4).—Compounds 2 (2.00 g, 3.57 mmol) and 3 (2.27 g, 3.75 mmol) were pre-dried in one flask under vacuum at 0°C for 4 h. The mixture was then dissolved in CH2Cl2 (20 mL). To the solution was added Me3SiOTf (40 μL, 0.22 mmol) under an N2 atmosphere at 0°C. The mixture was stirred at this temperature for 1 h, then neutralized with triethylamine, concentrated under reduced pressure, and purified on a silica gel column with 3:1 petroleum ether–EtOAc as the eluent to give 4 (3.44 g, 84.7%) as a syrup: [z]D's +74° (c 5.4, CHCl3); 1H NMR (CDCl3): δ 0.01 (s, 3 H, SiCH3), 0.16 (s, 3 H, SiCHOH), 0.74 (s, 9 H, C(CH3)2), 1.31 (d, 3 H, CH3), 1.41 (d, 3 H, CH3), 3.12 (m, 1 H, CH), 3.99 (q, 1 H, J3,4 2.4 Hz, H-3), 4.15–4.38 (m, 3 H, J6a,6b,6,10.8, J6a,5 2.6, J6b,5 6.7 Hz, H-6a, H-6b, H-5), 4.44 (br t, 1 H, J1, J1′,6b,6′ = 6.3 Hz, H-6′), 4.52 (q, 1 H, J6a,6b′ 11.0 Hz, H-6aa′), 4.65 (q, 1 H, H-6b′), 4.80 (d, 1 H, J1, J3,6 9.8 Hz, H-1), 5.08 (d, 1 H, J1, J1′,7.8 Hz, H-1′), 5.64 (br t, 1 H, J1, J2,7.6 Hz, H-2), 5.72 (dd, 1 H, J3,4,3.0 Hz, H-3′), 5.78 (dd, 1 H, J1, H-4), 5.96 (t, 1 H, J1, J2,10.0 Hz, H-2′), 6.13 (d, 1 H, H-4′), 7.37–8.29 (m, 30 H, PhCO). Anal. Caled for C63H86O16Si: C, 66.43; H, 5.80. Found: C, 66.32; H, 6.08.

Isopropyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,4-di-O-benzoyl-1-thio-β-D-galactopyranoside (5).—A solution of compound 4 (2.50 g, 2.20 mmol) in 90%aq trifluoroacetic acid (10 mL) was stirred at rt for about 1 h, then co-evaporated with toluene under diminished pressure to give a residue. Purification of the product by column chromatography (3:1 petroleum ether–EtOAc) gave 5 (1.72 g, 76.4%) as a syrup: [z]D's +45° (c 1, CHCl3); 1H NMR (CDCl3): δ 1.01 (d, 3 H, CH3), 1.06 (d, 3 H, CH3), 2.98 (m, 1 H, CH), 3.75–3.94 (m, 3 J6a,6a 10.8, J6a,5 2.6, J6b,5 6.7 Hz, H-6a, H-6b, H-5), 4.04 (q, 1 H, J3,4 2.4 Hz, H-3), 4.10–4.25 (m, 2 H, J1, J6a,6b 6.3, J6b,5 6.0, J6a,6b′ 11.0 Hz, H-6a′, H-5′), 4.32 (q, 1 H, H-6b′), 4.11 (d, 1 H, J1, J3,6 9.8 Hz, H-1), 4.78 (d, 1 H, J1, J2,7.8 Hz, H-1′), 5.22 (br t, 1 H, J1, J3,4 8.2 Hz, H-2′), 5.45 (dd, 1 H, J3,4,2.9 Hz, H-3′), 5.59 (d, 1 H, H-4), 5.68 (t, 1 H, J2,3,9.9 Hz, H-2′), 5.84 (d, 1 H, H-4′), 7.11–8.01 (m, 30 H, PhCO). Anal. Caled for C57H72O16S: C, 66.80; H, 5.09. Found: C, 66.87; H, 5.20.

Isopropyl 2,3,4,6-tetra-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,5-tri-O-benzoyl-x-L-arabinofuranosyl-(1→3)-2,4-di-O-benzoyl-1-thio-β-D-galactopyranosyl (7).—To a mixture of compound 5 (1.50 g, 1.46 mmol) and 6 (0.933 g, 1.54 mmol) in anhyd CH2Cl2 (15 mL) was added Me3SiOTf (26 μL, 0.15 mmol) under an N2 atmosphere at 0°C. The mixture was stirred under these conditions for 1 h, at which time TLC (2:1 petroleum ether–EtOAc) indicated that all starting materials were consumed. The reaction mixture was neutralized with Et3N, then concentrated. Column chromatography (2:1 petroleum ether–EtOAc) of the residue gave 7 (1.68 g, 78%) as a syrup: [z]D's +69° (c 5.0, CHCl3); 1H NMR (CDCl3): δ 1.08 (d, 3 H, CH3), 1.17 (d, 3 H, CH3), 3.06 (m, 1 H, CH), 3.78 (q, 1 H, J6a,6b,11.8 Hz, H-6a′), 4.08–4.15 (m, 2 H, J6a,6b 2.7, J6b,5 5.8 Hz, H-6′, H-5′), 4.18–4.23 (m, 3 H, J3,4,3.4, J5,6a 6.3, J5,6b 6.0 Hz, H-3′, H-6a′, H-5′), 4.36 (q, 1 H, J6a,6b,10.8 Hz, H-6b′), 4.46 (d, 1 H, J1, J1,2 10.0 Hz, H-1′), 4.70 (dd, 1 H, J4,5a 3.7, J4,5a,5b 12.2 Hz, H-5a′), 4.84 (m, 1 H, J1, J3,4 5.7 Hz, H-4′), 4.83 (d, 1 H, J1, J2,7.9 Hz, H-1′), 4.93 (dd, 1 H, J4,5b 2.8 Hz, H-5b), 5.25 (br s, 1 H, H-2′), 5.31 (s, 1 H, H-1′,H-5′), 5.48 (br d, 1 H, J4,4.5 5.5 Hz, H-3′), 5.52 (dd, 1 H, J3,4 3.4 Hz, H-4′), 5.66 (t, 1 H, J2,3,7.9 Hz, H-2′), 5.88 (d, 1 H, J3,4 3.4 Hz, H-4′), 5.92 (d, 1 H, J3,4,3.4 Hz, H-4′), 7.21–8.09 (m, 45 H, PhCO); 13C NMR (100 MHz, CDCl3): δ 23.39 (CH3), 23.78 (CH3), 35.00 (CH), 61.68 (C-6′), 63.27 (C-5′), 67.93 (C-4′), 68.19 (C-6′), 69.68
Preparation of methyl 2-O-benzyloxy-1-thio-D-galactopyranoside (9). —To the solution of phenyl 1-thio-D-galactopyranoside (2.20 g, 8.09 mmol) in pyridine (8 mL) was added tert-butylchloridophenylsilyl (2.7 mL, 28.4 mmol) as a syrup: [α]D20 + 97° (C 11.1, CHCl3); 1H NMR (300 MHz, CDCl3): δ 1.01 (s, 9 H, C(CH3)3), 3.79 (q, 1 H, J6a,6b = 10.1, J6a,5 = 7.7 Hz, H-6a), 3.90 (q, 1 H, J6b,5 = 6.1 Hz, H-6b), 4.14 (br t, 1 H, H-5), 4.98 (d, 1 H, J1,2 = 9.5 Hz, H-1), 5.61 (dd, 1 H, J3,4 = 2.9 Hz, H-3), 5.68 (t, 1 H, J2,3 = 9.8 Hz, H-2), 6.06 (d, 1 H, H-4), 7.21−7.97 (m, 30 H, PhCO). Anal. Calcd for C49H46O8SSi: C, 71.53; H, 5.60. Found: C, 71.45; H, 5.71.

Preparation of methyl 2-O-benzyloxy-4,6-O-isopropylidene-α-D-galactopyranoside (11). —To a solution of methyl 4,6-O-isopropylidene-α-D-galactopyranoside8 (7.00 g, 29.9 mmol) in pyridine (25 mL) was added benzoyl chloride (3.8 mL, 32.7 mmmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred at 50−60°C for 5 h, then poured into cold water, and extracted with CH2Cl2 (2 × 50 mL). The organic layer was dried and concentrated. Column chromatography (3:1 petroleum ether−EtOAc) of the residue gave syrupy 11 (4.20 g, 41.6%): [α]D20 + 135° (c 1.5, CHCl3); 1H NMR (CDCl3): δ 1.53 (s, 3 H, CH3), 1.64 (s, 3 H, CH3), 3.48 (s, 3 H, OCH3), 3.79 (d, 2 H, J6a,6b < 1, J6a,5 = J6b,5 = 6.4 Hz, H-6a, H-6b), 4.18 (br t, 1 H, J4,5 = 1.7 Hz, H-5), 4.33 (dd, 1 H, H-4), 4.55 (dd, 1 H, J3,a = 6.8 Hz, H-3), 5.00 (d, 1 H, J1,2 = 3.4 Hz, H-1), 5.14 (dd, 1 H, J3,4 = 8.1 Hz, H-2), 7.27−8.11 (m, 5 H, PhCO). Anal. Calcd for C83H72O23S: C, 67.85; H, 4.90. Found: C, 67.91; H, 5.01.

Preparation of methyl 2-O-benzyloxy-α-D-galactopyranoside (12). —Compound 11 (2.90 g, 8.85 mmol) was dissolved in 90% ac tri-fluoroacetic acid (8 mL). The solution was stirred for 30 min and co-evaporated with toluene to dryness under diminished pressure. The residue was purified on a silica gel column using EtOAc as eluent to give 12 (2.50 g, 97.6%) as crystals: mp 158−159°C; [α]D20 + 143° (c 2.1, water), lit.9 mp 171−173°C; [α]D20 + 170° (c 1, MeOH); 1H NMR (300 MHz, CDCl3): δ 3.30 (s, 3 H, OCH3), 3.78−4.10 (br m, 5 H, H-5, H-6a, H-6b, H-5 II, H-4), 4.96 (d, 1 H, J1,2 = 3.3 Hz, H-1), 5.22 (dd, 1 H, J2,3 = 6.6Hz, H-2), 7.24−8.01 (m, 5 H, PhCO).

Methyl 2-O-benzyloxy-6-O-tert-butyldiphenylsilyl-α-D-galactopyranoside (13). —To a solution of compound 12 (2.50 g, 8.39 mmol) in pyridine (10 mL) was added TBDPSCl (2.80 mL, 10.08 mmol) with a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred at rt overnight, and then poured into cold water, extracted with CH2Cl2 (2 × 50 mL), and the organic layer was dried over Na2SO4 and concentrated. Purification of the product by column chromatography (2:1 petroleum ether−EtOAc) gave 13 (4.05 g, 90%) as a syrup: [α]D20 + 36° (c 2.9, CHCl3); 1H NMR (300 MHz, CDCl3): δ 0.99 (s, 9 H, C(CH3)3), 3.23 (s, 3 H, OCH3), 3.77 (q, 1 H, J6b,5 = 5.2, J6a,5 = 4.8 Hz, H-5), 3.82−3.93 (br m, 2 H, J3,4 = 9.9 Hz, H-6b, H-6a), 4.03 (dd, 1 H, J3,4 = 9.9 Hz, H-3), 4.12 (d, 1 H, J3,4 = 3.3 Hz, H-4), 4.93 (d, 1 H, J1,2 = 3.6 Hz, H-1), 5.16 (dd, 1 H, H-2), 7.16−8.00 (m, 15 H, PhCO). Anal. Calcd for C172H152O34Si: C, 67.16; H, 6.72. Found: C, 67.10; H, 6.81.

Methyl 2,3,5-tri-O-benzyloxy-α-L-arabinofuranosyl-(1→3)-2-O-benzyloxy-6-O-tert-butyldiphenylsilyl-α-D-galactopyranoside (14). —To a mixture of compound 13 (2.50 g, 4.46 mmol) and 6 (2.97 g, 4.90 mmol) in anhyd CH2Cl2 (20 mL) was added Me3SiOTf (42 μL, 0.23 mmol) under an N2 atmosphere at 0°C. The
mixture was stirred under these conditions for 1 h, neutralized with Et₃N, and then concentrated. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave syrup 14 (3.28 g, 71.8%): [α]$_D^{20} + 19^\circ$ (c 0.3, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.96 (s, 9 H, C(CH$_3$)$_3$), 3.23 (s, 3 H, OCH$_3$), 3.75–3.94 (br d, 3 H, $J_{6b,5} = J_{6a,5} = 5.1$), $J_{6b,6a} < 1$ Hz, H-5, H-6b, H-6a), 4.17 (d, 1 H, J$_{3,4} 3.0$ Hz, H-4), 4.29 (dd, 1 H, J$_{2,3} 10.2$ Hz, H-3), 4.48–4.64 (m, 2 H, J$_{4a,5a} 10.0$, J$_{4',5'a} 4.8$, J$_{4',5'b}$ 5.4 Hz, H-4', H-5a'), 4.70 (dd, 1 H, J$_{1,2} 3.6$ Hz, H-1), 5.34 (dd, 1 H, J$_{2,3} 10.2$ Hz, H-2), 5.41 (br s, 1 H, H-2'), 5.49 (d, 1 H, J$_{3,4}$ 3.9 Hz, H-3'), 5.52 (s, 1 H, H-1'), 7.06–8.10 (m, 30 H, PhCO). Anal. Calcd for C$_{56}$H$_{56}$O$_{14}$Si: C, 68.57; H, 5.71. Found: C, 68.49; H, 5.90.

*Methyl 2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→3)-2-O-benzoyl-α-D-galactopyranoside (15).—A solution of compound 14 (3.00 g, 3.06 mmol) in 90% aq trifluoroacetic acid (15 mL) was stirred at rt for 1 h, then neutralized with NaHCO$_3$ and extracted with CH$_2$Cl$_2$ (3 x 50 mL). The organic phases were combined and concentrated. The residue was purified on a silica gel column using 3:2 petroleum ether–EtOAc as eluent to give syrup 15 (1.94 g, 85.5%): [α]$_D^{20} + 81^\circ$ (c 1.8, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 3.31 (s, 3 H, OCH$_3$), 3.70–4.00 (br m, 3 H, J$_{6a,5} = J_{6b,5} = 9.9$ Hz, H-5, H-6b, H-6a), 4.22 (d, 1 H, J$_{3,4}$ 3.0 Hz, H-4), 4.35 (dd, 1 H, J$_{3,2}$ 9.9 Hz, H-3), 4.52–4.68 (br m, 2 H, J$_{4a,5a} 10.0$, J$_{4',5'a} 4.8$, J$_{4',5'b}$ 5.4 Hz, H-4', H-5a'), 4.74 (dd, 1 H, J$_{1,2}$ 3.3 Hz, H-1), 5.10 (d, 1 H, J$_{1,2}$ 3.3 Hz, H-2'), 5.44 (br s, 1 H, H-2'), 5.51 (br d, 1 H, J$_{3,4}$ 3.9 Hz, H-3'), 5.54 (s, 1 H, H-1'), 7.16–8.00 (m, 30 H, PhCO). Anal. Calcd for C$_{83}$H$_{78}$O$_{22}$Si: C, 68.50; H, 5.36. Found: C, 68.33; H, 5.62.

*Methyl 2,3,4-tri-O-benzoyl-6-O-tert-butyl-diphenyldisilyl-β-D-galactopyranosyl-(1→6)-[2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1→3)]-2-O-benzoyl-α-D-galactopyranoside (16).—To a solution of compound 15 (1.68 g, 2.26 mmol) and 9 (1.96 g, 2.38 mmol) in anhyd CH$_2$Cl$_2$ (15 mL) was added NIS (1.34 g, 5.96 mmol) and Me$_3$SiOTf (120 µL, 0.66 mmol) under an N$_2$ atmosphere at 0 °C. The mixture was stirred under these conditions for 1 h, at which time TLC (5:2 petroleum ether–EtOAc) indicated that starting material 15 was completely consumed. The reaction mixture was neutralized with Et$_3$N, then concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave 16 (2.31 g, 70.2%) as a syrup: [α]$_D^{20} + 97^\circ$ (c 1.6, CHCl$_3$); $^1$H NMR (CDCl$_3$): $\delta$ 0.97 (s, 9 H, C(CH$_3$)$_3$), 2.87 (s, 3 H, OCH$_3$), 3.80–3.90 (m, 3 H, H-6a', H-6b', H-6a''), 3.97 (br d, 1 H, J$_{5,6a} 8.3$ Hz, H-5'), 4.07–4.13 (m, 3 H, H-4', H-5', H-6b''), 4.28 (dd, 1 H, J$_{3,4} 3.4$ Hz, H-3'), 4.59–4.64 (m, 2 H, J$_{4,5,9} 3.9$, J$_{5a,5b} 12.1$ Hz, H-5a''', H-4a'''), 4.73 (q, 1 H, J$_{4,5,b} 2.7$ Hz, H-5b'''), 4.80 (d, 1 H, J$_{1,2} 3.6$ Hz, H-1'), 4.85 (d, 1 H, J$_{1,2} 7.8$ Hz, H-1'''), 5.32 (dd, 1 H, J$_{2,3} 10.4$ Hz, H-2'), 5.45 (br s, 1 H, H-2''''), 5.51 (s, 1 H, H-1'''''), 5.53 (br d, 1 H, J$_{3,4} 3.0$ Hz, H-3'''''), 5.63 (dd, 1 H, J$_{3,4} 3.3$ Hz, H-3'''''), 5.72 (dd, 1 H, J$_{2,3} 10.4$ Hz, H-2''''), 6.07 (d, 1 H, H-4'''''), 7.07–8.03 (m, 45 H, PhCO). Anal. Calcd for C$_{83}$H$_{78}$O$_{22}$Si: C, 68.50; H, 5.36. Found: C, 68.33; H, 5.62.
Methyl 2,3,4-tri-O-benzoyl-β-D-galactopyranosyl-(1 → 6)-[2,3,5-tri-O-benzoyl-α-L-arabinofuranosyl-(1 → 3)]-4-O-acetyl-2-O-benzoyl-α-D-galactopyranoside (19).—To a mixture of compound 18 (600 mg, 0.477 mmol) and 7 (735 mg, 0.501 mmol) in anhyd CH₂Cl₂ (10 mL) was added NIS (282 mg, 1.25 mmol) and Me₃SiOTf (47 μL, 0.26 mmol) under an N₂ atmosphere at −15 °C. The mixture was stirred under these conditions for 1 h, at which time TLC (5:2 petroleum ether–EtOAc) indicated the completion of the reaction. The reaction mixture was neutralized with Et₃N, and concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave 19 (1.05 g, 83.1%) as a syrup: [α]D²⁰ +61° (c 3.4, CHCl₃); ¹H NMR (CDCl₃): δ 1.86 (s, 3 H, CH₃CO); 13C NMR (CDCl₃): δ 60.75 (C-5I), 65.70 (C-6I), 67.50 (C-5II), 68.85 (C-5III), 69.28 (C-6II), 69.68 (C-6III), 70.42 (C-3I), 71.18 (C-3II), 71.48 (C-3III), 74.52 (C-4I), 77.10 (C-4II), 81.29 (C-4III), 81.56 (C-4IV), 84.70 (C-5IV), 87.56 (C-3IV), 90.49 (C-4V), 92.39 (C-3V), 92.84 (C-4V), 94.92 (C-5V), 103.70 (C-1I), 109.94 (C-1II), 110.93 (C-1III), 113.79 (C-1IV), 119.45 (C-1V), 121.71 (C-2I), 151.48 (C-2II), 165.67–166.63 (C 3, PhCO), 170.24 (CH₃CO); MALDI-TOF MS Caled for C₁₄₉H₁₂₆O₄₆: 2650 [M]; Found: C₁₄₉H₁₂₆O₄₆: C, 67.47; H, 4.75. Found: C, 67.30; H, 5.19.
Methyl β-D-galactopyranosyl-(1 → 6)-[α-L-arabinofuranosyl-(1 → 3)]-β-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-(1 → 6)[α-L-arabinofuranosyl-(1 → 3)]-α-D-galactopyranoside (20). — Ammonia was bubbled into a mixture of 19 (1.05 g, 0.369 mmol) in anhyd MeOH (150 mL) at 4°C until saturation. The mixture was kept at rt for about 7 days and then evaporated to dryness. Purification on a Sephadex LH-20 column with MeOH as eluent furnished 20 (365 mg, 97.6%) as an amorphous solid: [α]D20 0 − 11° (c 0.5, water); 1H NMR (MeOD): δ 3.35 (s, 3 H, OCH3), 3.50–4.20 (m, 34 H), 4.31–4.34 (br t, 2 H, J 7.3, J 5.7 Hz, H-1III, H-1IV), 4.40 (d, 1 H, J 7.1 Hz, H-1II), 4.72 (d, 1 H, J 3.6 Hz, H-1I), 5.19 (s, 1 H, H-1VI), 5.24 (s, 1 H, H-1V); 13C NMR (100 Hz, MeOD): δ 55.93 (1 C, OCH3), 62.55 (1 C), 63.33 (2 C), 69.13 (1 C), 69.83–70.41 (8 C), 71.74 (1 C), 72.41 (1 C), 72.49 (1 C), 74.56 (1 C), 74.84 (1 C), 75.16 (2 C), 76.64 (1 C), 77.99 (1 C), 78.97 (1 C), 79.30 (1 C), 80.93 (1 C), 82.84 (1 C), 82.92 (1 C), 86.66 (1 C), 86.69 (1 C), 101.53 (C-1I), 105.04 (C-1IV), 105.43 (2 C, C-1II, C-1III), 111.11 (C-1VI), 111.17 (C-1V); ESIMS Calcd for C35H60O29: 944 [M]; Found ESIMS (negative-ion): 979 [M + NH4OH]−, ESIMS (positive-ion): 967 [M + Na]+.

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References