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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-galactopyranoside, 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.

Keywords: Carbohydrate; Arabinogalactan; Regioselective reaction; Antigen; Epitope

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.[1] As part of our ongoing research project on the synthesis of epitopes related to arabinogalactan proteins (AGPs),[2] 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.[3]

There has been no unambiguous structural definition on CCRC-M7-recognized arabinogalactan epitopes so far reported.[3–5] 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.[5] 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[6] 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-

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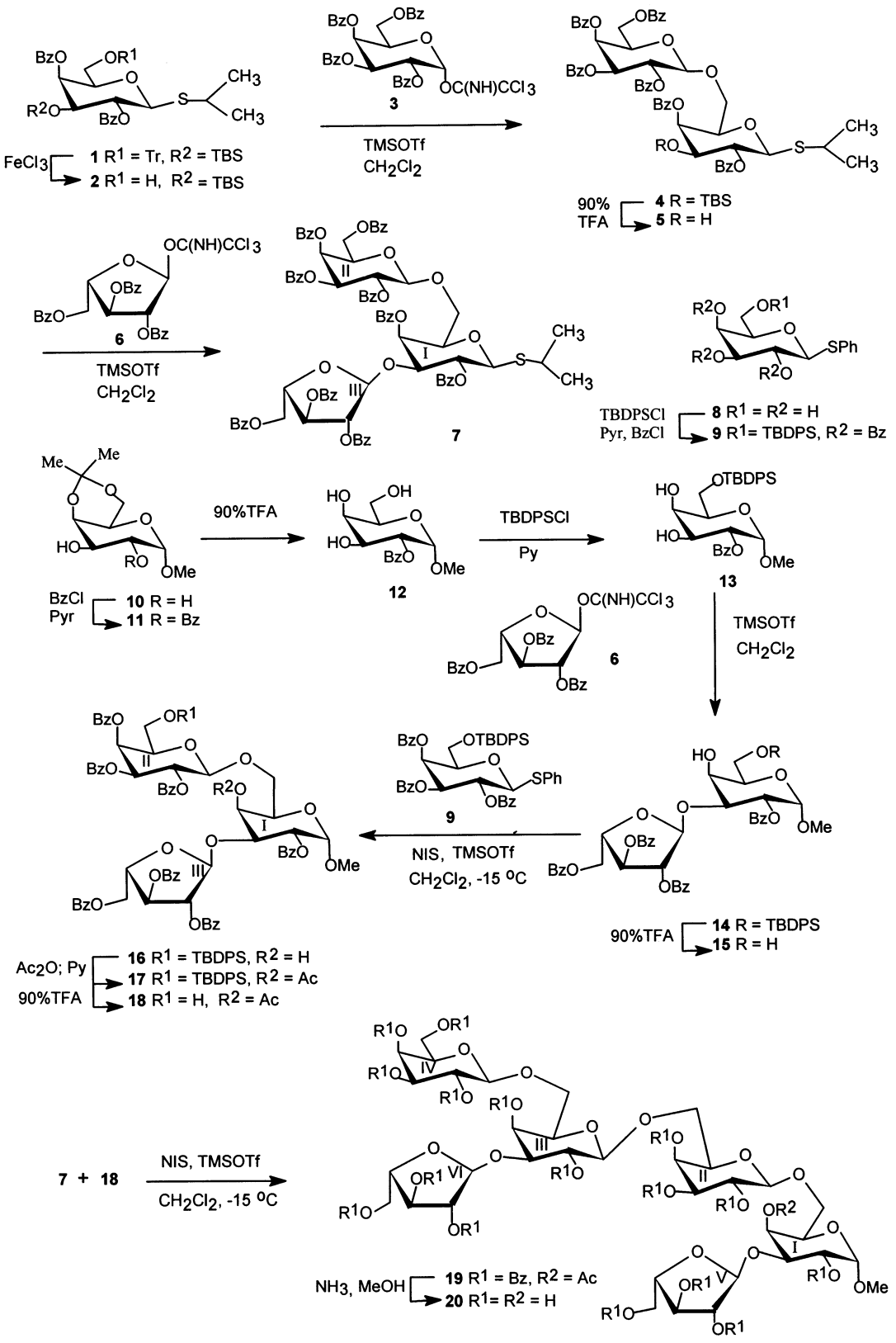
galactopyranoside (**1**) was prepared from commercially available isopropyl 1-thio-β-D-galactopyranoside (IPTG) according to our previous method.[6] $FeCl_3$ hexahydrate catalyzed detritylation[7] was carried out smoothly on compound **1** providing the 6-OH acceptor **2** in 85.5% yield. Standard glycosylation of **2** with fully benzoylated galactopyranosyl imidate **3** in anhydrous CH_2Cl_2 gave (1→6)-linked di-galactopyranoside **4**, followed by desilylation with 90% trifluroacetic 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 benzoylation 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 ^1H 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_2Cl_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 ^1H–^1H COSY spectroscopy. Desilylation of **14** in 90% TFA (→**15**), followed by NIS–TMSOTf catalyzed glycosylation with **9** in anhydrous CH_2Cl_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 ammonia-saturated methanol completed the synthesis of the target compound, 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**), 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. ^1H, ^{13}C NMR and ^1H–^1H, ^1H–^{13}C COSY spectra were recorded with ARX 400 spectrometers for solutions in $CDCl_3$, MeOD and D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si, or DSS in the case of D_2O. 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_2SO_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**). —Ferric trichloride hexahydrate (2.0 equiv) was added to a mixture of **1** (4.24 g, 5.29 mmol) in dry CH_2Cl_2 (30 mL). The mixture was stirred at rt for 3 h, then diluted with more CH_2Cl_2 (50 mL), and washed twice with ice-cold water. The washings were re-extracted with CH_2Cl_2 (20 mL). The combined organic


FeCl3
1 R1 = Tr, R2 = TBS
2 R1 = H, R2 = TBS
3
TMSOTf
CH2Cl2
90%
TFA
4 R = TBS
5 R = H
6
TMSOTf
CH2Cl2
7
TBDPSCl
Pyr, BzCl
8 R1 = R2 = H
9 R1= TBDPS, R2 = Bz
90%TFA
BzCl
Pyr
10 R = H
11 R = Bz
12
TBDPSCl
Py
13
TMSOTf
CH2Cl2
NIS, TMSOTf
CH2Cl2, -15 °C
14 R = TBDPS
15 R = H
90%TFA
Ac2O; Py
90%TFA
16 R1 = TBDPS, R2 = H
17 R1 = TBDPS, R2 = Ac
18 R1 = H, R2 = Ac
7 + 18
NIS, TMSOTf
CH2Cl2, -15 °C
NH3, MeOH
19 R1 = Bz, R2 = Ac
20 R1= R2 = H

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; $[\alpha]_D^{20}$ + 76° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.01 (s, 3 H, SiCH_3), 0.16 (s, 3 H, SiCH_3), 0.74 (s, 9 H, C(CH_3)$_3$), 1.41 (d, 3 H, CH_3), 1.44 (d, 3 H, CH_3), 3.39 (m, 1 H, C*H*), 3.72 (q, 1 H, $J_{6a,6b}$11.1, $J_{6a,5}$ 6.3 Hz, H-6a), 3.93 (q, 1 H, $J_{6b,5}$ 5.7 Hz, H-6b), 4.02 (t, 1 H, H-5), 4.28 (d, 1 H, $J_{3,4}$ < 1.0 Hz, H-3), 4.88 (d, 1 H, $J_{1,2}$ 9.9 Hz, H-1), 5.62 (d, 1 H, H-4), 5.73 (t, 1 H, $J_{2,3}$ 9.6 Hz, H-2), 7.58–8.28 (m, 10 H, *Ph*CO). Anal. Calcd for $C_{29}H_{40}O_7SSi$: 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-tert-*butyldimethylsilyl-1-thio-β*-D-*galactopyranoside* (**4**).—Compounds **2** (2.00 g, 3.57 mmol) and **3** (2.27 g, 3.75 mmol) were predried in one flask under vacuum at 60 °C for 4 h. The mixture was then dissolved in CH_2Cl_2 (20 mL). To the solution was added Me_3SiOTf (40 μL, 0.22 mmol) under an N_2 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: $[\alpha]_D^{20}$ + 74° (*c* 5.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.01 (s, 3 H, SiCH_3), 0.16 (s, 3 H, SiCH_3), 0.74 (s, 9 H, C(CH_3)$_3$), 1.31 (d, 3 H, CH_3), 1.41 (d, 3 H, CH_3), 3.12 (m, 1 H, C*H*), 3.99 (q, 1 H, $J_{3,4}$ 2.4 Hz, H-3), 4.15–4.38 (m, 3 H, $J_{6a,6b}$10.8, $J_{6a,5}$ 2.6, $J_{6b,5}$ 6.7 Hz, H-6a, H-6b, H-5), 4.44 (br t, 1 H, $J_{5',6b'} = J_{5',6a'} = 6.3$ Hz, H-5′), 4.52 (q, 1 H, $J_{6a',6b'}$11.0 Hz, H-6a′), 4.65 (q, 1 H, H-6b′), 4.80 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 5.08 (d, 1 H, $J_{1',2'}$7.8 Hz, H-1′), 5.64 (br t, 1 H, $J_{2,3}$7.6 Hz, H-2), 5.72 (dd, 1 H, $J_{3',4'}$3.0 Hz, H-3′), 5.78 (d, 1 H, H-4), 5.96 (t, 1 H, $J_{2',3'}$10.0 Hz, H-2′), 6.13 (d, 1 H, H-4′), 7.37–8.29 (m, 30 H, *Ph*CO). Anal. Calcd for $C_{63}H_{66}O_{16}SSi$: 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 trifluroacetic 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: $[\alpha]_D^{20}$ + 45° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.01 (d, 3 H, CH_3), 1.06 (d, 3 H, CH_3), 2.98 (m, 1 H, C*H*), 3.75–3.94 (m, 3 H, $J_{6a,6b}$10.8, $J_{6a,5}$ 2.6, $J_{6b,5}$ 6.7 Hz, H-6a, H-6b, H-5), 4.04 (q, 1 H, $J_{3,4}$ 2.4 Hz, H-3), 4.10–4.25 (m, 2 H, $J_{5',6a'}$ 6.3, $J_{6b',5'}$ 6.0, $J_{6a',6b'}$ 11.0 Hz, H-6a′, H-5′), 4.32 (q, 1 H, H-6b′), 4.11 (d, 1 H, $J_{1,2}$ 9.8 Hz, H-1), 4.78 (d, 1 H, $J_{1',2'}$7.8 Hz, H-1′), 5.22 (br t, 1 H, $J_{2,3}$ 8.2 Hz, H-2), 5.45 (dd, 1 H, $J_{3',4'}$2.9 Hz, H-3′), 5.59 (d, 1 H, H-4), 5.68 (t, 1 H, $J_{2',3'}$9.9 Hz, H-2′), 5.84 (d, 1 H, H-4′), 7.11–8.01 (m, 30 H, *Ph*CO). Anal. Calcd for $C_{57}H_{52}O_{16}S$: 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-α*-L-*arabinofuranosyl-(1→3)]-2,4-di*-O-*benzoyl-1-thio-β*-D-*galactopyranoside* (**7**).—To a mixture of compound **5** (1.50 g, 1.46 mmol) and **6** (0.933 g, 1.54 mmol) in anhyd CH_2Cl_2 (15 mL) was added Me_3SiOTf (26 μL, 0.15 mmol) under an N_2 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 Et_3N, then concentrated. Column chromatography (2:1 petroleum ether–EtOAc) of the residue gave **7** (1.68 g, 78%) as a syrup: $[\alpha]_D^{20}$ + 69° (*c* 5.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.08 (d, 3 H, CH_3), 1.17 (d, 3 H, CH_3), 3.06 (m, 1 H, C*H*), 3.78 (q, 1 H, $J_{6a,6b}$11.8 Hz, H-6a^I), 4.08–4.15 (m, 2 H, $J_{6a,5}$ 2.7, $J_{6b,5}$ 5.8 Hz, H-6b^I, H-5^I), 4.18–4.23 (m, 3 H, $J_{3,4}$ 3.4, $J_{5,6a}$ 6.3, $J_{5,6b}$ 6.0 Hz, H-3^I, H-6a^{II}, H-5^{II}), 4.36 (q, 1 H, $J_{6a,6b}$10.8 Hz, H-6b^{II}), 4.66 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1^I), 4.70 (dd, 1 H, $J_{4,5a}$ 3.7, $J_{5a,5b}$ 12.2 Hz, H-5a^{III}), 4.84 (m, 1 H, $J_{4,3}$ 5.7 Hz, H-4^{III}), 4.87 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^{II}), 4.93 (dd, 1 H, $J_{4,5b}$ 2.8 Hz, H-5b^{III}), 5.25 (br s, 1 H, H-2^{III}), 5.31 (s, 1 H, H-1^{III}), 5.48 (br d, 1 H, $J_{3,4}$ 5.5 Hz, H-3^{III}), 5.52 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3^{II}), 5.66 (t, 1 H, $J_{2,3}$ 9.8 Hz, H-2^{II}), 5.77 (dd, 1 H, $J_{2,3}$ 7.9 Hz, H-2^I), 5.88 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^I), 5.92 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^{II}), 7.21–8.09 (m, 45 H, *Ph*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.39 (*C*H$_3$), 23.78 (*C*H$_3$), 35.00 (*C*H), 61.68 (C-6^{II}), 63.27 (C-5^{III}), 67.93 (C-4^{II}), 68.19 (C-6^I), 69.68

(C-2^{I}), 70.18 (C-2^{II}), 70.58 (C-4^{I}), 71.15 (C-3^{I}), 71.73 (C-3^{II}), 77.10 (C-5^{I}), 77.54 (C-3^{III}), 77.66 (C-5^{II}), 81.57 (C-4^{III}), 82.52 (C-2^{III}), 83.56 (C-1^{I}), 100.99 (C-1^{II}), 107.67 (C-1^{III}), 164.61–166.11 (9 C, Ph*C*O); MALDI-TOF MS Calcd for $C_{83}H_{72}O_{23}S$: 1468 [M]; Found 1491.6 $[M + Na]^+$, 1507.6 $[M + K]^+$. Anal. Calcd for $C_{83}H_{72}O_{23}S$: C, 67.85; H, 4.90. Found: C, 67.91; H, 5.01.

Phenyl 2,3,4-tri-O-*benzoyl-6*-O-tert-*butyldiphenylsilyl-1-thio-β*-D-*galactopyranoside* (**9**).—To the solution of phenyl 1-thio-β-D-galactopyranoside (2.20 g, 8.09 mmol) in pyridine (8 mL) was added *tert*-butylchlorodiphenylsilane (2.7 mL, 9.72 mmol). The mixture was stirred at rt for 16 h, at which time TLC (2:1 petroleum ether–EtOAc) indicated that all starting materials were consumed. Then benzoyl chloride (3.30 mL, 28.4 mmol) was added dropwise. The mixture was stirred at rt for 10 h, then co-evaporated with toluene to remove pyridine under diminished pressure. The residue was purified on a silica gel column using 3:1 petroleum ether–EtOAc as eluent to give **9** (4.95 g, 74.4%) as a syrup: $[\alpha]_D^{20}$ + 97° (*c* 11.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.01 (s, 9 H, C(CH_3)$_3$), 3.79 (q, 1 H, $J_{6a,6b}$10.1, $J_{6a,5}$ 7.7 Hz, H-6a), 3.90 (q, 1 H, $J_{6b,5}$ 6.1 Hz, H-6b), 4.14 (br t, 1 H, H-5), 4.98 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.61 (dd, 1 H, $J_{3,4}$ 2.9 Hz, H-3), 5.68 (t, 1 H, $J_{2,3}$ 9.8 Hz, H-2), 6.0.6 (d, 1 H, H-4), 7.21–7.97 (m, 30 H, *Ph*CO). Anal. Calcd for $C_{49}H_{46}O_8SSi$: C, 71.53; H, 5.60. Found: C, 71.45; H, 5.71.

Preparation of methyl 2-O-*benzoyl-4,6*-O-*isopropylidene-α*-D-*galactopyranoside* (**11**).—To a solution of methyl 4,6-*O*-isopropylidene-α-D-galactopyranoside[8] (7.00 g, 29.9 mmol) in pyridine (25 mL) was added benzoyl chloride (3.8 mL, 32.7 mmol) 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 CH_2Cl_2 (2 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated. Column chromatography (3:1 petroleum ether–EtOAc) of the residue gave syrupy **11** (4.20 g, 41.6%): $[\alpha]_D^{20}$ + 135° (*c* 1.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.53 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 3.48 (s, 3 H, OCH_3), 3.79 (d, 2 H, $J_{6a,6b} < 1$, $J_{6a,5} = J_{6b,5} = 6.4$ Hz, H-6a, H-6b), 4.18 (br t, 1 H, $J_{4,5}$1.7 Hz, H-5), 4.33 (dd, 1 H, H-4), 4.55 (dd, 1 H, $J_{3,4}$ 6.8 Hz, H-3), 5.00 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.14 (dd, 1 H, $J_{2,3}$ 8.1 Hz, H-2), 7.27–8.11 (m, 5 H, *Ph*CO). Anal. Calcd for $C_{17}H_{22}O_7$: C, 60.36; H, 6.51. Found: C, 60.40; H, 7.01.

Preparation of methyl 2-O-*benzoyl-α*-D-*galactopyranoside* (**12**).—Compound **11** (2.90 g, 8.85 mmol) was dissolved in 90% aq trifluroacetic acid (8 mL). The solution was stirred at rt 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; $[\alpha]_D^{20}$ + 143° (*c* 2.1, water), lit.[9] mp 171–173 °C; $[\alpha]_D^{20}$ + 170° (*c* 1, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.30 (s, 3 H, OCH_3), 3.78–4.10 (br m, 5 H, $J_{6a,6b}$10.2, $J_{2,3}$ 6.6 Hz, H-6a, H-6b, H-5, H-4, H-3), 4.96 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.22 (dd, 1 H, $J_{2,3}$ 6.6Hz, H-2), 7.24–8.01 (m, 5 H, *Ph*CO).

Methyl 2-O-*benzoyl-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 CH_2Cl_2 (2 × 50 mL), and the organic layer was dried over Na_2SO_4 and concentrated. Purification of the product by column chromatography (2:1 petroleum ether–EtOAc) gave **13** (4.05 g, 90%) as a syrup: $[\alpha]_D^{20}$ + 36° (*c* 2.9, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (s, 9 H, C(CH_3)$_3$), 3.23 (s, 3 H, OCH_3), 3.77 (q, 1 H, $J_{6b,5}$ 5.2, $J_{6a,5}$ 4.8 Hz, H-5), 3.82–3.93 (br m, 2 H, $J_{6b,6a}$10.8 Hz, H-6b, H-6a), 4.03 (dd, 1 H, $J_{3,2}$ 9.9 Hz, H-3), 4.12 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-4), 4.93 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.16 (dd, 1 H, H-2), 7.16–8.00 (m, 15 H, *Ph*CO). Anal. Calcd for $C_{30}H_{36}O_7Si$: C, 67.16; H, 6.72. Found: C, 67.10; H, 6.81.

Methyl 2,3,5-tri-O-*benzoyl-α*-L-*arabinofuranosyl-(1→3)-2*-O-*benzoyl-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 CH_2Cl_2 (20 mL) was added Me_3SiOTf (42 μL, 0.23 mmol) under an N_2 atmosphere at 0 °C. The

mixture was stirred under these conditions for 1 h, neutralized with Et_3N, and then concentrated. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave syrupy **14** (3.28 g, 71.8%): $[\alpha]_D^{20}$ +19° (*c* 0.3, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 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, $J_{5a',5b'}$ 10.0, $J_{4',5a'}$ 4.8, $J_{4',5b'}$ 5.4 Hz, H-4′, H-5a′), 4.70 (dd, 1 H, H-5b′), 5.09 (d, 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, *Ph*CO). 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 trifluroacetic acid (15 mL) was stirred at rt for 1 h, then neutralized with $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 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 syrupy **15** (1.94 g, 85.5%): $[\alpha]_D^{20}$ +81° (*c* 1.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 3.31 (s, 3 H, OCH_3), 3.70–4.00 (br m, 3 H, $J_{6b,5} = J_{6a,5} = 5.1$, $J_{6b,6a}$ 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_{5a',5b'}$10.0, $J_{4',5a'}$ 4.8, $J_{4',5b'}$ 5.4 Hz, H-4′, H-5a′), 4.74 (dd, 1 H, H-5b′), 5.10 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.40 (dd, 1 H, $J_{2,3}$ 9.9 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, *Ph*CO). Anal. Calcd for $C_{40}H_{38}O_{14}$: C, 64.69; H, 5.12. Found: C, 64.60; H, 5.22.

Methyl 2,3,4-tri-O-*benzoyl*-*6*-O-tert-*butyldiphenylsilyl*-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_2Cl_2 (15 mL) was added NIS (1.34 g, 5.96 mmol) and Me_3SiOTf (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_3N, then concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **16** (2.31 g, 70.2%) as a syrup: $[\alpha]_D^{20}$ +97° (*c* 1.6, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 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^I, H-6b^I, H-6a^{II}), 3.97 (br d, 1 H, $J_{5,6a}$ 8.3 Hz, H-5^I), 4.07–4.13 (m, 3 H, H-4^I, H-5^{II}, H-6b^{II}), 4.28 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3^I), 4.59–4.64 (m, 2 H, $J_{4,5a}$ 3.9, $J_{5a,5b}$ 12.1 Hz, H-5a^{III}, H-4^{III}), 4.73 (q, 1 H, $J_{4,5b}$ 2.7 Hz, H-5b^{III}), 4.80 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^I), 4.85 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 5.32 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2^I), 5.45 (br s, 1 H, H-2^{III}), 5.51 (s, 1 H, H-1^{III}), 5.53 (br d, 1 H, $J_{3,4}$ 3.0 Hz, H-3^{III}), 5.63 (dd, 1 H, $J_{3,4}$ 3.3 Hz, H-3^{II}), 5.72 (dd, 1 H, $J_{2,3}$ 10.4 Hz, H-2^{II}), 6.07 (d, 1 H, H-4^{II}), 7.07–8.03 (m, 45 H, *Ph*CO). 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-*butyldiphenylsilyl*-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* (**17**).—To a solution of compound **16** (1.42 g, 0.979 mmol) in pyridine (4 mL) was added Ac_2O (2 mL). The mixture was stirred at rt for about 20 h, then co-evaporated with toluene under diminished pressure to remove pyridine. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **17** (1.22 g, 83.6%) as a syrup: $[\alpha]_D^{20}$ +115° (*c* 3.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.00 (s, 9 H, $C(CH_3)_3$), 1.87 (s, 3 H, CH_3CO), 2.92 (s, 3 H, OCH_3), 3.61 (q, 1 H, $J_{6a,6b}$ 10.6 Hz, H-6a^I), 3.78–3.85 (m, 2 H, $J_{6a,6b}$ 13.1 Hz, H-6a^{II}, H-6b^{II}), 3.94 (q, 1 H, $J_{5,6b}$ 2.5 Hz, H-6b^I), 4.07 (t, 1 H, $J_{6b,5} = J_{6a,5} = 7.4$ Hz, H-5^{II}), 4.14 (br d, 1 H, $J_{5,6a}$ 8.0 Hz, H-5^I), 4.44 (dd, 1 H, $J_{3,4}$ 3.4 Hz, H-3^I), 4.67 (dd, 1 H, $J_{4,5a}$ 3.9, $J_{5a,5b}$ 12.1 Hz, H-5a^{III}), 4.78 (m, 1 H, H-4^{III}), 4.83 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{II}), 4.85 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^I), 4.90 (dd, 1 H, $J_{4,5b}$ 2.7 Hz, H-5b^{III}), 5.28 (dd, 1 H, $J_{2,3}$ 10.6 Hz, H-2^I), 5.36 (br s, 1 H, H-2^{III}), 5.45 (s, 1 H, H-1^{III}), 5.48–5.54 (br d, 2 H, $J_{3,4}$ 5.0 Hz, H-4^I, H-3^{III}), 5.62 (dd, 1 H, $J_{3,4}$3.3 Hz, H-3^{II}), 5.69 (dd, 1 H, $J_{2,3}$10.4 Hz, H-2^{II}), 6.06 (d, 1 H,

H-4^{II}), 7.09–8.06 (m, 45 H, *Ph*CO). Anal. Calcd for $C_{85}H_{80}O_{23}Si$: C, 68.18; H, 5.35. Found: C, 68.31; H, 5.23.

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* (**18**).—A solution of compound **17** (1.00 g, 0.668 mmol) in 90% aq trifluroacetic acid (10 mL) was stirred at rt for 3 h, at which time TLC (3:2 petroleum ether–EtOAc) indicated the completion of the reaction. The mixture was co-evaporated with toluene under diminished pressure to dryness and purified on a silica gel column with 3:2 petroleum ether–EtOAc as the eluent to furnish **18** (651 mg, 77.4%) as a syrup: $[\alpha]_D^{20}$ +178° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.94 (s, 3 H, CH_3CO), 2.99 (s, 3 H, OCH_3), 3.64–3.68 (m, 2 H, H-6a^I, H-6a^{II}), 3.85 (dd, 1 H, $J_{6a,6b}$11.9 Hz, H-6b^{II}), 3.96 (dd, 1 H, $J_{6a,6b}$9.3 Hz, H-6b^I), 4.07 (t, 1 H, $J_{6b,5} = J_{6a,5}$ = 7.0 Hz, H-5^{II}), 4.18 (dd, 1 H, $J_{5,6a} = J_{5,6b} = 4.6$ Hz, H-5^I), 4.46 (dd, 1 H, $J_{3,4}$3.4 Hz, H-3^I), 4.68 (dd, 1 H, $J_{4,5a}$ 3.9, $J_{5a,5b}$ 12.1 Hz, H-5a^{III}), 4.78 (m, 1 H, H-4^{III}), 4.85–4.87 (q, 2 H, $J_{1A,2A}$ 3.6, $J_{1B,2B}$ 7.8 Hz, H-1^I, H-1^{II}), 4.93 (dd, 1 H, $J_{4,5b}$ 2.8 Hz, H-5b^{III}), 5.31 (dd, 1 H, $J_{2,3}$ 10.5 Hz, H-2^I), 5.36 (s, 1 H, H-2^{III}), 5.46 (s, 1 H, H-1^{III}), 5.51 (br d, 1 H, $J_{3,4}$ 3.7 Hz, H-3^{III}), 5.57 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4^I), 5.58 (dd, 1 H, $J_{3,4}$ 3.3, $J_{2,3}$10.6 Hz, H-3^{II}), 5.82–5.87 (m, 2 H, H-2^{II}, H-4^{II}), 7.22–8.10 (m, 35 H, *Ph*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.70 (*C*H_3CO), 55.02 (O*C*H$_3$), 60.54 (C-6^{II}), 63.30 (C-5^{III}), 68.38 (C-5^I), 68.94 (C-4^{II}), 69.28 (C-6^I), 69.95 (C-2^{II}), 70.98 (C-4^I), 71.24 (C-2^I), 71.71 (C-3^{II}), 71.95 (C-3^I), 74.04 (C-5^{II}), 77.86 (C-3^{III}), 81.29 (C-4^{III}), 82.26 (C-2^{III}), 97.07 (C-1^I), 101.92 (C-1^{II}), 107.54 (C-1^{III}), 164.78–166.83 (7 C, Ph*C*O), 170.24 (CH$_3$*C*O); MALDI-TOF MS Calcd for $C_{69}H_{62}O_{23}$: 1258 [M]; Found 1281.4 [M + Na], 1297.3 [M + K]. Anal. Calcd for $C_{69}H_{62}O_{23}$: C, 65.82; H, 4.93. Found: C, 65.70; H, 5.17.

Methyl 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*-β-D-*galactopyranosyl*-(1→6)-*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 CH_2Cl_2 (10 mL) was added NIS (282 mg, 1.25 mmol) and Me_3SiOTf (47 μL, 0.26 mmol) under an N_2 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 Et_3N, and concentrated. Column chromatography (5:2 petroleum ether–EtOAc) of the residue gave **19** (1.05 g, 83.1%) as a syrup: $[\alpha]_D^{20}$ +61° (*c* 3.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.86 (s, 3 H, CH_3CO), 2.93 (s, 3 H, OCH_3), 3.33 (dd, 1 H, $J_{5,6b}$ 2.71, $J_{6a,6b}$ 11.8 Hz, H-6a^{III}), 3.58 (dd, 1 H, $J_{6a,6b}$ 9.3, $J_{5,6a}$ 8.0 Hz, H-6a^I), 3.75–3.92 (m, 5 H, H-6a^{II}, H-6b^{II}, H-5^{II}, H-6a^{IV}, H-6b^I), 3.95 (dd, 1 H, $J_{5,6b}$ 5.8 Hz, H-6b^{III}), 4.02–4.15 (m, 5 H, H-3^{III}, H-5^{III}, H-5^{IV}, H-6b^{IV}, H-5^I), 4.44 (dd, 1 H, $J_{2,3}$ 10.5, $J_{3,4}$ 3.4 Hz, H-3^I), 4.51 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1^{III}), 4.54 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1^{IV}), 4.66 (dd, 1 H, $J_{4,5a}$ 3.9, $J_{5a,5b}$ 12.1 Hz, H-5a^V), 4.72–4.85 (br, 4 H, H-1^I, H-1^{II}, H-5a^{VI}, H-4^V), 4.91 (dd, 1 H, $J_{4,5b}$ 2.8 Hz, H-5b^V), 4.98–5.05 (br, 2 H, H-5b^{VI}, H-4^{VI}), 5.24–5.29 (m, 3 H, H-1^{VI}, H-2^{VI}, H-2^I), 5.36 (s, 1 H, H-2^V), 5.44 (s, 1 H, H-1^V), 5.46–5.62 (m, 6 H, H-2^{III}, H-3^{IV}, H-3^{VI}, H-4^I, H-3^{II}, H-3^V), 5.64–5.68 (m, 2 H, H-2^{IV}, H-2^{II}), 5.81–5.84 (m, 2 H, H-4^{III}, H-4^{IV}), 5.96 (d, 1 H, H-4^{II}), 7.21–8.21 (m, 80 H, *Ph*CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.61 (*C*H_3CO), 54.89 (O*C*H$_3$), 61.22 (C-6^{IV}), 63.22 (C-5^V), 63.37 (C-5^{VI}), 66.36 (C-6^{III}), 66.46 (C-6^{II}), 67.75 (C-4^{IV}), 67.99 (C-4^{II}), 68.28 (C-5^I), 68.69 (C-6^I), 69.44 (C-4^{III}), 69.87 (C-2^{IV}), 69.92 (C-2^{II}), 70.76 (C-3^{IV}), 70.90 (C-3^{III}), 71.24 (C-2^I), 71.61 (C-3^{II}), 71.68 (2 C, C-2^{III}, C-4^I), 71.92 (C-3^I), 72.40 (C-5^{III}), 72.57 (C-5^{II}), 76.40 (C-5^{IV}), 77.65 (C-3^V), 77.81 (C-3^{VI}), 81.18 (C-4^V), 81.64 (C-4^{VI}), 82.28 (C-2^V), 82.61 (C-2^{VI}), 97.93 (C-1^I), 100.79 (C-1^{IV}), 100.94 (C-1^{III}), 101.60 (C-1^{II}), 107.50 (C-1^V), 107.68 (C-1^{VI}), 164.62–166.18 (16 C, Ph*C*O), 169.91 (CH$_3$*C*O); MALDI-TOF MS Calcd for $C_{149}H_{126}O_{46}$: 2650 [M]; Found 2673.6 [M + Na]$^+$. Anal. Calcd for $C_{149}H_{126}O_{46}$: 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: $[\alpha]_D^{20}$ −11° (*c* 0.5, water); ^{1}H NMR (MeOD): δ 3.35 (s, 3 H, OCH_3), 3.50–4.20 (m, 34 H), 4.31–4.34 (br t, 2 H, *J* 7.3, *J* 5.7 Hz, H-1^{III}, H-1^{IV}), 4.40 (d, 1 H, *J* 7.1 Hz, H-1^{II}), 4.72 (d, 1 H, *J* 3.6 Hz, H-1^{I}), 5.19 (s, 1 H, H-1^{VI}), 5.24 (s, 1 H, H-1^{V}); ^{13}C NMR (100 Hz, MeOD): δ 55.93 (1 C, OCH$_3$), 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-1^{I}), 105.04 (C-1^{IV}), 105.43 (2 C, C-1^{II}, C-1^{III}), 111.11 (C-1^{VI}), 111.17 (C-1^{V}); ESIMS Calcd for $C_{35}H_{60}O_{29}$: 944 [M]; Found ESIMS (negative-ion): 979 $[M + NH_4OH]^-$, ESIMS (positive-ion): 967 $[M + Na]^+$.

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