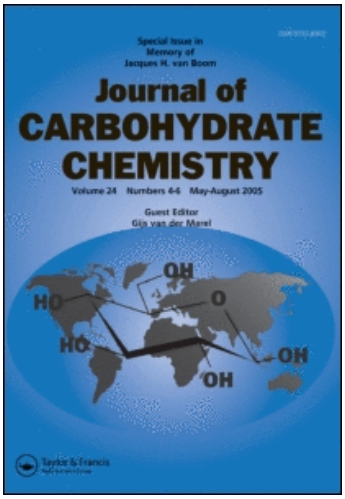

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SYNTHESIS OF 3-*O*-ARABINOSYLATED (1→6)-β-D-GALACTAN EPITOPES

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ABSTRACT

Two tetrameric arabinogalactans, β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→3)]-D-galactopyranose (**14**) and α-L-arabinofuranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactopyranose (**25**), which are good candidates for CCRC-M7 epitope characterization, were synthesized efficiently using a convergent strategy. Migration of an acceptor acetyl group proved to be an obstacle to synthesis, but regioselective glycosylation or 4-*O*-benzyl protection of the acceptor circumvented this problem allowing efficient synthesis of the 1→6 linked target compounds.

INTRODUCTION

As part of an ongoing research effort on arabinogalactan-protein (AGP) related epitopes, we have synthesized dodecyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→2)]-β-D-galactopyranoside.[1] The synthesis of a 2-*O*-arabinofuranosylated tetrameric arabinogalactan modeled on 1,2-anhydrosugars has been reported by van Boom's group.[2] The epitope of this arabinogalactan recognized by the CCRC-M7 antibody consists of a β-(1→6)-linked galactan containing at least three D-galactopyranosyl residues functionalized at one hydroxyl group with an α-(1→3)-linked L-arabinofuranosyl unit corresponding to a proposal of Albersheim.[3] Although structure and activity

relationship studies have shown the importance of the arabinofuranose side chain and its potential pharmacological activity,[4] we do not yet have a complete structural knowledge of these AGP epitopes.[5] We present here the assembly of two tetrameric arabinogalactans, which represent the minimal AGP epitope units. We believe these will prove extremely useful for antibody screening, immunogen development and inhibitor construction.[6]

RESULTS AND DISCUSSION

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-6-*O*-triphenylmethyl-1,2-*O*-ethylidene-α-D-galactopyranose (**4**) was prepared by regioselective coupling of 6-*O*-triphenylmethyl-1,2-*O*-ethylidene-α-D-galactopyranose (**2**) with *O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl) trichloroacetimidate (**3**) under standard glycosylation conditions. Acetylation of **4** with acetic anhydride in pyridine gave **5** for which the chemical shift of H-4 was found to be at δ 5.64 ppm, thus confirming the 1→3 linkage in **4**. Detritylation of **5** using ferric chloride hexahydrate in dichloromethane[7] was complicated by partial acetyl migration which occurred under these conditions. Nevertheless, the desired 4-*O*-acetylated product **6** was obtained in 48% yield. The reaction was followed by TLC. The acetyl migrated (6-*O*-acetylated) byproduct **7** was found to be formed from the very beginning of the reaction process and the amount of **7** continued to increase with reaction time. Glycosylation of **6** with disaccharide donor 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**10**)[1] in CH_2Cl_2 using TMSOTf as the catalyst furnished 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-[2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)]-4-*O*-acetyl-1,2-*O*-ethylidene-α-D-galactopyranose (**11**) in 51% yield. Alternatively, removal of the triphenylmethyl ether group of **4** gave the 4,6-diol **8** in high yield. Regioselective glycosylation of **8** with **10** provided the tetrasaccharide **12** in 87% yield. Acetylation of **12** resulted in a product with the same NMR spectrum as **11** confirming the structural assignment of **12**. Trifluoroacetic acid promoted hydrolysis of the 1,2-*O*-ethylidene group in **12** followed by acetylation with acetic anhydride in pyridine gave the fully acylated tetrasaccharide **13**. Treatment of **13** with a catalytic amount of NaOMe in methanol provided the target compound **14** in good yield (70% from **12**).

A similar synthetic strategy was also applied to the construction of the AG derivative **25**. Thus, **8** was acetylated, and then deacetalated using 90% trifluroacetic acid to give **15**. Acetylation of **15** with acetic anhydride in pyridine (→ **16**) followed by regioselective deacetylation of the anomeric carbon using benzylamine (→ **17**),[8] and activation with trichloroacetonitrile using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) furnished 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate (**18**) in good yield. In order to prevent undesired acetyl migration, 4-*O*-benzylation was carried out on the acceptor. Therefore compound **19**[1] was treated with methanolic sodium methoxide (→**20**), regioselectively silylated with TBDPSCl in pyridine

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(→**21**) and benzylated with benzyl bromide and sodium hydride in DMF furnishing **22** in 77.6% yield (from **19**). Tetra-*n*-butylammonium fluoride (TBAF)-mediated desilylation of **22** and condensation of the primary hydroxyl in **23** with imidate **18** led to the tetrameric derivative **24** in 85% yield. Treatment of **24** with 95% TFA to remove the ethylidene group, acetylation with acetic anhydride in pyridine, hydrogenolysis using H_2 and Pd/C in MeOH/EtOAc, and finally deprotection by Zemplén deacylation, provided the free tetrasaccharide **25** in a total yield of 49% (from **24**).

EXPERIMENTAL

General Methods. Optical rotations were determined at 25°C with a Perkin-Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY spectra were recorded with ARX 400 spectrometers for solutions in $CDCl_3$, CD_3OD and D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were recorded with a VG PLATFORM mass spectrometer using 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 (10×240 mm, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc - petroleum ether (60–90°C) as the eluent. Solutions were concentrated at < 60°C under diminished pressure. The (*R* and *S*) configuration assignment for the ethylidene group in oligosaccharides was based on Kochetkov's report.[9] Pure *R* or *S* isomers were obtained by column chromatography. However, the isomers underwent isomerization during long term storage. There was no difference in the reactivity for the two isomers.

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-6-*O*-triphenylmethyl-1,2-*O*-ethylidene-α-D-galactopyranose (4). To a solution of **1**[9] (3.4 g, 16.5 mmol) in pyridine (10 mL) was added TrCl (5.51 g, 19.8 mmol). The mixture was stirred at rt overnight, then poured into cold water, extracted with CH_2Cl_2 (2 × 30 mL), and the organic layer was dried over Na_2SO_4, and concentrated. Column chromatography (2/1 petroleum ether-ethyl acetate) of the residue gave **2** as a syrup (*R*,*S*-mixture, 4.46 g, 61%). To a solution of **2** (2.11 g, 4.71 mmol) and **3** (2.806 g, 4.63 mmol) in anhydrous CH_2Cl_2 (25 mL) was added trimethylsilyl triflate (30 μL, 0.17 mmol) in the presence of 4 Å molecular sieves under a nitrogen atmosphere at −40°C. The mixture was stirred at rt for 2 h, at the end of which time TLC (2/1 petroleum ether-ethyl acetate) indicated that all starting material **3** was consumed. The reaction mixture was neutralized with triethylamine, then filtered and the filtrate was concentrated. Purification of the product by column chromatography (2:1 petroleum ether-ethyl acetate) gave syrupy **4** (*R*,*S*-mixture, 2.60 g, 63%); For *R*-isomer, $[\alpha]_D^{25}$ −7° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (d, 3 H, C*H*$_3$CH), 3.00 (br t, 1 H, J 7.0 Hz, H-6a), 3.34 (dd, 1 H, $J_{5,6a}$ 5.8, $J_{6a,6b}$ 7.0 Hz, H-6b), 4.03–4.05 (m, 2 H, H-2, H-4), 4.30 (br t, 1 H, J 7.0 Hz, H-5),

4.73 (dd, 1 H, J 3.6, 11.6 Hz, H-5′a), 4.86–4.92 (m, 2 H, H-4′, H-5′b), 5.18 (q, 1 H, J 4.9 Hz, CH_3CH), 5.45 (d, 1 H, J 3.6 Hz, H-1), 5.56 (s, 1 H, H-2′/H-1′), 5.58 (d, 1 H, H-3′), 5.60 (s, 1 H, H-1′/H-2′), 7.21–8.12 (m, 30 H, Ph).

Anal. Calcd for $C_{53}H_{48}O_{13}$: C, 71.30; H, 5.38. Found C, 71.46; H, 5.19 (*R*,*S* mixture).

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-4-*O*-acetyl-6-*O*-triphenylmethyl-1,2-*O*-ethylidene-α-D-galactopyranose (5). Compound **4** (2.54 g, 2.85 mmol) was acetylated with acetic anhydride (2 mL) in pyridine (5 mL) under standard conditions to give **5** (2.53 g, 95%) as a syrup; For *R*-isomer, $[\alpha]_D^{25}$ +3° (*c* 0.6, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.46 (d, 3 H, CH_3CH), 1.71 (s, 3 H, CH_3CO), 3.00 (dd, 1 H, $J_{5,6a}$ 8.0, $J_{6a,6b}$ 9.0 Hz, H-6a), 3.35 (dd, 1 H, $J_{5,6b}$ 7.0 Hz, H-6b), 4.03–4.05 (m, 2 H, H-2, H-3), 4.28–4.32 (m, 1 H, H-5), 4.73 (dd, 1 H, $J_{2,3}$ 11.3, $J_{3,4}$ 3.2 Hz, H-5′a), 4.86–4.94 (m, 2 H, H-5′b, H-4′), 5.18 (q, 1 H, J 4.8 Hz, CH_3CH), 5.45 (d, 1 H, J 3.8 Hz, H-1), 5.54 (s, 1 H, H-1′), 5.60 (d, 1 H, J 1.6 Hz, H-3′), 5.61 (s, 1 H, H-2′), 5.64 (br s, 1 H, H-4), 7.22–8.12 (m, 30 H, Ph).

Anal. Calcd for $C_{55}H_{50}O_{14}$: C, 70.66; H, 5.35. Found C, 70.72; H, 5.40 (*R*,*S* mixture).

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-4-*O*-acetyl-1,2-*O*-ethylidene-α-D-galactopyranose (6) and 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-6-*O*-acetyl-1,2-*O*-ethylidene-α-D-galactopyranose (7). To a solution of **5** (934 mg, 1.0 mmol) in CH_2Cl_2 (20 mL) was added ferric chloride hexahydrate (670 mg, 2.5 mmol). The mixture was stirred at rt for 4 h, at the end of which time TLC (2/1 petroleum ether-ethyl acetate) showed the reaction was complete. The mixture was poured into cold water and extracted with dichloromethane. The organic phase was washed with saturated $NaHCO_3$, then dried over Na_2SO_4 and concentrated. Column chromatography (2/1 petroleum ether-ethyl acetate) of the residue gave **6** (304 mg, 48%), **7** (101 mg, 16%), and recovered starting material **5** (300 mg). For *S*-isomer of **6**, $[\alpha]_D^{25}$ +18° (*c* 2.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.38 (d, 3 H, J 4.8 Hz, CH_3CH), 2.00 (s, 3 H, CH_3CO), 3.45 (dd, 1 H, $J_{5,6a}$ 4.4, $J_{6a,6b}$ 9 Hz, H-6a), 3.69 (dd, 1 H, $J_{5,6b}$ 6.0, $J_{6a,6b}$ 9.0 Hz, H-6b), 4.05–4.15 (m, 2 H, H-2, H-5), 4.38 (dd, 1 H, J 4.8, 7.6 Hz, H-3), 4.63–4.71 (m, 2 H, H-4, H-5′a), 4.81–4.85 (m, 1 H, H-5′b), 5.45 (d, 1 H, J 2.8 Hz, H-4), 5.49 (q, 1 H, CH_3CH), 5.50–5.65 (m, 4 H, H-1, H-1′, 2′, 3′), 7.26–8.08 (m, 15 H, Ph).

Anal. Calcd for $C_{36}H_{36}O_{14}$: C, 62.43; H, 5.20. Found: C, 62.31; H, 5.33.

For compound **7** (*R*:*S* = 1:2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.36 (d, 2 H, CH_3CH), 1.45 (d, 1 H, CH_3CH), 1.99 (s, 1 H, CH_3CO), 2.07 (s, 2 H, CH_3CO), 4.02–4.16 (m, 4 H, H-2,5,6a,6b), 4.30–4.39 (m, 2 H, H-3, H-4), 4.68–4.83 (m, 3 H, H-4′, H-5′a, H-5′b), 5.14 (q, 0.33 H, H-1(*S*)), 5.60–5.68 (m, 3.67 H, H-1′,2′,3′, H-1 (*R*)), 7.25–8.11 (m, 15 H, Ph). ESMS Calcd for $C_{36}H_{36}O_{14}$ (692.2), Found ESI-MS(+) 710.4 $(M+NH_4)^+$.

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-1,2-*O*-ethylidene-α-D-galacto-pyranose (8). To a solution of **4** (2.54 g, 2.85 mmol) in CH_2Cl_2 (50 mL) was added ferric chloride hexahydrate (1.68 g, 6.3 mmol) as described in the prepa-

ration of **6** to give **8** as a syrup (*R*,*S*-mixture, 1.518 g, 82%); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.34 (d, 1.5 H, C*H*$_3$CH), 1.45 (d, 1.5 H, C*H*$_3$CH), 3.80–4.21 (m, 5.5 H, H-2, H-4, H-5, H-6a, H-6b and H-3 (*R* or *S*)), 4.40 (br t, 0.5 H, J 6.2 Hz, H-3 (*S* or *R*)), 4.65–4.81 (m, 3 H, H-4′, H-5′a, H-5′b), 5.14 (q, 0.5 H, J 4.8 Hz, CH_3C*H* (S)), 5.48 (q, 0.5 H, J 4.8 Hz, CH_3C*H* (*R*)), 5.60–5.67 (m, 3 H, H-1′, H-2′, H-3′), 7.25–8.07 (m, 15 H, Ph). ESMS Calcd for: $C_{34}H_{34}O_{13}$ (650.2). Found ESI-MS(+) 673.3 $(M+Na)^+$.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galacto-pyranosyl-(1→6)-[2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)]-4-*O*-acetyl-1,2-*O*-ethylidene-α-D-galactopyranose (11). To a solution of **6** (240 mg, 0.35 mmol) and **10** (271 mg, 0.35 mmol) in anhydrous CH_2Cl_2 (10 mL) was added trimethylsilyl triflate (11 μL, 0.06 mmol) under a nitrogen atmosphere at −15°C. The mixture was stirred under this condition for 2 h, at the end of which time TLC (1/1 petroleum ether-ethyl acetate) indicated that the starting material **10** was completely consumed. The reaction mixture was neutralized with triethylamine, then filtered and the filtrate was concentrated. Purification of the product by column chromatography (3:2 petroleum ether-ethyl acetate) gave **11** as a syrup (234 mg, 51%); For *S*-isomer, $[\alpha]_D^{25}$ +8° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.36 (d, 3 H, J 4.6 Hz, C*H*$_3$CH), 1.93, 1.99, 2.01, 2.07, 2.11, 2.13 (6 s, 24 H, 8 C*H*$_3$CO), 3.69–3.89 (m, 6 H), 4.01–4.15 (m, 4 H), 4.37 (m, 1 H), 4.54 (d, 1 H, J 9.0 Hz, H-1^B), 4.58 (d, 1 H, J 8.5 Hz, H-1^C), 4.68–4.71 (m, 2 H, H-4^D, H-5a^D), 4.90 (dd, 1 H, J 4.2, 8.5 Hz, H-5b^D), 4.85–5.00 (m, 4 H), 5.37 (br s, 2 H, H-4^B, H-4^C), 5.45 (q, 1 H, CH_3C*H*), 5.53–5.60 (m, 5 H, H-4^A,H-1^D,2^D,3^D, H-1^A), 7.26–8.10 (m, 15 H, Ph). ESMS Calcd for $C_{62}H_{70}O_{31}$ (1310); Found ESI-MS(+) 1333 $(M+Na)^+$.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galacto-pyranosyl-(1→6)-[2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)]-1,2-*O*-ethylidene-α-D-galactopyranose (12). To a solution of **8** (680 mg, 1.05 mmol) and **10** (871 mg, 1.11 mmol) in anhydrous CH_2Cl_2 (15 mL) was added trimethylsilyl triflate (20 μL, 0.11 mmol) in the presence of 4 Å molecular sieves under a nitrogen atmosphere at −40°C. The mixture was stirred under this condition for 2 h, at the end of which time TLC (1/1 petroleum ether-ethyl acetate) indicated that the starting material **10** was completely consumed. The reaction mixture was neutralized with triethylamine, then filtered and the filtrate was concentrated. Purification of the product by column chromatography (3:2 petroleum ether-ethyl acetate) gave **12** as a syrup (1.158 g, 87%); For *S*-isomer, $[\alpha]_D^{25}$ +22° (*c* 4.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.36 (d, 3 H, J 4.6 Hz, C*H*$_3$CH), 1.97, 1.98, 2.04, 2.07, 2.11, 2.13, 2.14 (7 s, 21 H, 7 C*H*$_3$CO), 3.66–3.90 (m, 6 H), 4.01–4.15 (m, 5 H), 4.32 (br t, 1 H, J 6.6 Hz), 4.49 (d, 1 H, J 9.1 Hz, H-1^B), 4.58 (d, 1 H, J 8.5 Hz, H-1^C), 4.68–4.71 (m, 2 H, H-4^D, H-5a^D), 4.90 (dd, 1 H, J 3.6, 9.2 Hz, H-5b^D), 4.85–5.00 (m, 4 H), 5.37 (br s, 2 H, H-4*B*, H-4*C*), 5.45 (q, 1 H, CH_3C*H*), 5.53–5.60 (m, 4 H, H-1^D,2^D,3^D, H-1^A), 7.26–8.10 (m, 15 H, Ph). ESMS Calcd for $C_{60}H_{68}O_{30}$ (1268.4); Found ESI-MS(+) 1291.2 $(M+Na)^+$.

Anal. Calcd for $C_{60}H_{68}O_{30}$: C, 56.78; H, 5.36. Found : C, 56.59; H, 5.44.

β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→3)]-D-galactopyranose (14). Compound **12** (340 mg, 0.27 mmol) was dissolved in aqueous 90% TFA (5 mL) and the mixture was stirred at rt. The reaction was monitored by TLC (2/3 petroleum ether-ethyl acetate) until all of the starting material was consumed (about 40 min). The mixture was diluted with toluene and then concentrated to dryness. After acetylation of the residue with acetic anhydride (2 mL) in pyridine (5 mL), the solution was co-evaporated with toluene. Purification of the products on column chromatography (2/3 petroleum ether-ethyl acetate) gave **13** as a syrup which was subsequently treated with NaOMe in MeOH (keep pH at 9) at rt for 24 h, then neutralized with Dowex-50 (H^+) resin and, concentrated. The product was purified by Sephadex LH-20 column chromatography using methanol as the eluent to afford **14** (120 mg, 70% from **12**); $[\alpha]_D^{25}$ −48° (*c* 0.9, H_2O); ^{13}C NMR (D_2O, 100 MHz): δ 62.3 (C-6^C), 63.1 (C-5^D), 69.4, 69.5, 69.9, 71.5, 71.8, 72.6, 73.4, 73.8, 73.9, 74.0, 74.5, 74.8, 76.6, 78.2, 79.2, 82.1, 85.8, 103.0, 104.5, 104.7 (C-1^A/C-1^B/C-1^C), 111.2 (C-1^D). ESMS Calcd for $C_{23}H_{40}O_{20}$ (636.2). Found ESI-MS(+): 659.1 $(M+Na)^+$.

Anal. Calcd for $C_{23}H_{40}O_{20}$: C, 43.40; H, 6.29. Found: C, 43.25; H, 6.40.

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-4,6-di-*O*-acetyl-1,2-*O*-ethylidene-α-D-galactopyranose (9). Compound **8** (1.345 g, 2.07 mmol) was acetylated with acetic anhydride (3 mL) in pyridine (5 mL) under standard conditions to give **9** (1.49 g, 98%) as a syrup; For *R*-isomer, $[\alpha]_D^{25}$ +3° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.42 (d, 3 H, J 4.8 Hz, CH_3CH), 1.96, 2.05 (2 s, 6 H, 2 CH_3CO), 4.08–4.15 (m, 3 H, H-2, 2 H-6), 4.26–4.30 (m, 1 H, H-5), 4.34 (dd, 1 H, $J_{2,3}$ 7.1, $J_{3,4}$ 4.5 Hz, H-3), 4.68–4.76 (m, 2 H, H-4′, H-5′a), 4.89 (dd, 1 H, $J_{4',5'b}$ 2.7, $J_{5'a,5'b}$ 11.6 Hz, H-5′b), 5.56 (d, 1 H, $J_{1,2}$ 4.7 Hz, H-1), 5.57 (s, 1 H, H-1′/H-2′), 5.61–5.63 (d, 2 H, $J_{3',4'}$ 4.6 Hz, H-3′, H-2′/H-1′), 7.27–8.11 (m, 15 H, Ph).

Anal. Calcd for $C_{38}H_{38}O_{15}$: C, 62.12; H, 5.18. Found: C, 62.31; H, 5.15.

2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-galactopyra-nosyl 2, 2, 2-trichloroacetimidate (18). Compound **9** (2.2 g, 3 mmol) was dissolved in aqueous 90% trifluoroacetic acid (10 mL). The solution was stirred at rt for 3 h and then co-evaporated with toluene to dryness under reduced pressure. The syrup generated above was fully acetylated with acetic anhydride (4 mL) in pyridine (8 mL) for 4 h at room temperature, then co-evaporated with toluene (2 × 20 mL) to dryness. Column chromatography using 2/1 petroleum ether-ethyl acetate as the eluent gave syrupy **16** quantitatively. To a solution of **16** in THF (20 mL) was added benzylamine (3.4 mL, 31 mmol) under ice-water bath conditions. The solution was stirred at rt for 10 h, at the end of which time TLC (1/1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was poured into cold water and extracted with CH_2Cl_2. The organic phase was washed with 5% HCl (2 × 100 mL) and water (100 mL), then dried over Na_2SO_4 and concentrated. Column chromatography (1/1 petroleum ether-ethyl acetate) of the residue gave **17** as a syrup (1.69 g, 75%). To a solution of **17** (2.47 g, 3.3 mmol) in anhydrous CH_2Cl_2 (20 mL) were added trichloroacetonitrile (1.5 mL,

15 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene(1,5-5) (DBU, 0.2 mL), and the mixture was stirred at room temperature for 2 h. TLC (2/1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was concentrated and purified on a silica gel column using 2:1 petroleum ether-ethyl acetate as eluent to give **18** as a syrup (2.20 g, 74.8%); $[\alpha]_D^{25}$ −40° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.90, 2.00, 2.12 (3 s, 9 H, 3 C*H*$_3$CO), 4.04 (dd, 1 H, $J_{5,6a}$ 6.8, $J_{6a,6b}$ 10.1 Hz, H-6a), 4.10–4.20 (m, 2 H, H-3, H-6b), 4.32–4.42 (m, 1 H, H-5), 4.76 (dd, 1 H, J 3.6, J 11.9 Hz, H-5′a), 4.80–4.90 (m, 2 H, H-4, H-5′b), 4.90 (dd, 1 H, $J_{2,3}$ 12.0 Hz, H-2), 5.40, 5.42 (2 s, 2 H, H-1′, H-2′), 5.58 (d, 1 H, J 4.2 Hz, H-3′), 5.64 (d, 1 H, J 2.2 Hz, H-4), 6.60 (d, 1 H, $J_{1,2}$ 3.1 Hz, H-1), 7.23–8.14 (m, 15 H, Ph), 8.65 (s,1 H,N*H*).

Anal. Calcd for $C_{40}H_{38}Cl_3NO_{16}$: C, 53.66; H, 4.25. Found: C, 53.52; H, 4.41.

6-*O*-*tert*-Butyldiphenylsilyl-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (22). Compound **19** (4.5 g, 7.56 mmol) was treated with NaOMe in MeOH (keep pH at 9) at rt for 24 h, then neutralized with Dowex-50 (H^+) resin and concentrated. The crude product was dissolved in pyridine (20 mL) and *tert*-butyldiphenylsilyl chloride (2.5 g, 9 mmol) and 4-dimethylaminopyridine (30 mg) were added to the solution. The mixture was stirred at rt for 16 h, then concentrated under reduced pressure. Flash column chromatography (2/3 petroleum ether-ethyl acetate) of the residue gave crude **21** as a syrup. To a cooled solution of **21** in DMF (12 mL) was added NaH (2.17 g, 50%, 45 mmol) and BnBr (2.8 mL, 24.4 mmol). The mixture was stirred at rt for 6 h, at the end of which time TLC (3/1 petroleum ether-ethyl acetate) indicated that the reaction was complete. The mixture was poured into cold water and extracted with CH_2Cl_2. The organic phase was washed with water, then dried over Na_2SO_4 and concentrated. Column chromatography (4/1 petroleum ether-ethyl acetate) of the residue gave **22** as a syrup (5.29 g, 77.6% from **19**). $[\alpha]_D^{25}$ −56° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.04 (s, 9 H, C(C*H*$_3$)$_3$), 1.27, 1.29, 1.42, 1.46 (4 s, 12 H, 2 (C*H*$_3$)$_2$C), 3.36 (dd, 1 H, $J_{5,6a}$ 7.8, $J_{6a,6b}$ 9.2 Hz, H-6a), 3.50 (dd, 1 H, $J_{5',6'a}$ 2.8, $J_{6'a,6'b}$ 11.4 Hz, H-6′a), 3.62 (dd, 1 H, $J_{5',6'b}$ 7.6 Hz, H-6′b), 3.70–3.85 (m, 2 H, H-5′, H-6b), 3.92 (d, 1 H, J 2.7 Hz, H-4′), 4.01–4.10 (m, 2 H, H-3′, H-5), 4.15 (dd, 1 H, $J_{2,3}$ 2.4, $J_{3,4}$ 7.9 Hz, H-3), 4.28 (dd, 1 H, $J_{1,2}$ 5.0, $J_{2,3}$ 2.4 Hz, H-2), 4.34 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1′), 4.53 (dd, 1 H, $J_{3,4}$ 7.9, $J_{4,5}$ 2.4 Hz, H-4), 4.61 (d, 1 H, J 11.3 Hz, PhC*H*$_2$), 4.73 (d, 2 H, J 10.7 Hz, 2 PhC*H*$_2$), 4.83, 4.98, 5.03 (3 d, 3 H, J 11.3, 10.7, 10.7 Hz, 3 PhC*H*$_2$), 5.55 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 7.20–7.62 (m, 25 H, Ph).

Anal. Calcd for $C_{55}H_{66}O_{11}$: C, 73.17; H, 7.32. Found: C, 73.24; H, 7.20.

2,3,4-Tri-*O*-benzyl-β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (23). To a solution of **22** (840 mg, 0.93 mmol) in THF (20 mL) was added tetrabutylammonium fluoride trihydrate (300 mg, 0.95 mmol). The mixture was stirred at rt for 5 h, at the end of which time TLC (2/1 petroleum ether-ethyl acetate) showed the reaction was complete. The mixture was concentrated under reduced pressure. Column chromatography (2/1 petroleum

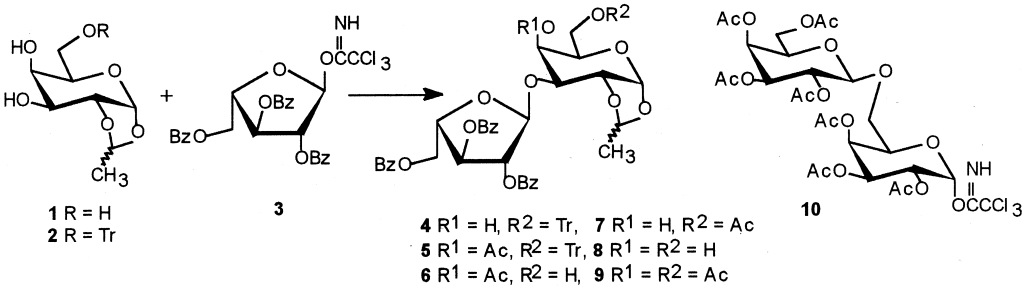
ether-ethyl acetate) of the residue gave **23** as a syrup (574 mg, 89%). $[\alpha]_D^{25}$ −6° (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.31, 1.32, 1.43, 1.50 (4 s, 12 H, 2 (CH_3)$_2$C), 3.36 (dd, 1 H, $J_{6'a,6'b}$ 5.2, $J_{5',6'b}$ 6.8 Hz, H-6′a), 3.42 (dd, 1 H, $J_{5',6'b}$ 11.1 Hz, H-6′b), 3.51 (dd, 1 H, $J_{5,6a}$ 2.9, $J_{6a,6b}$ 9.7 Hz, H-6a), 3.71–3.78 (m, 3 H, H-4′,5′,H-6b), 3.82 (dd, 1 H, $J_{1',2'}$ 7.7 Hz, $J_{2',3'}$ 10.0 Hz, H-2′), 4.03–4.05 (m, 1 H, H-5), 4.10 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 4.7 Hz, H-3′), 4.28 (dd, 1 H, $J_{2,3}$ 2.0 Hz, H-2), 4.41 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.60 (dd, 1 H, $J_{3,4}$ 7.9, $J_{4,5}$ 2.4 Hz, H-4), 4.65, 4.72, 4.75, 4.83, 4.93, 5.03 (6 d, 6 H, J 12.0, 11.7, 11.0 Hz, 3 PhCH_2), 5.55 (d, 1 H, $J_{1,2}$ 5.0 Hz, H-1), 7.25–7.45 (m, 15 H, Ph).

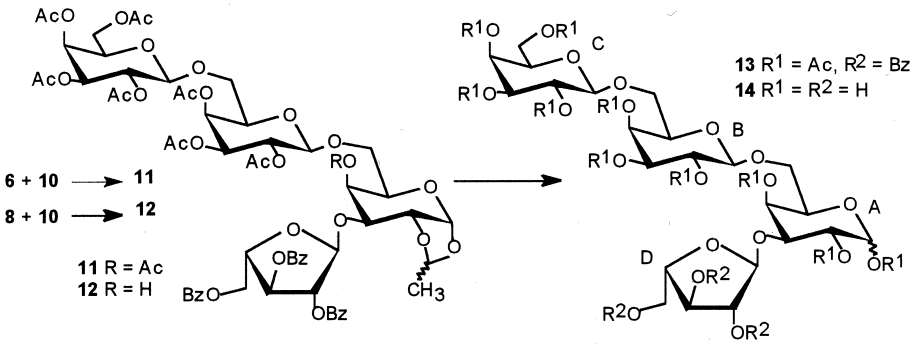
Anal. Calcd for $C_{39}H_{48}O_{11}$: C, 67.63; H, 6.94. Found: C, 67.55; H, 7.08.

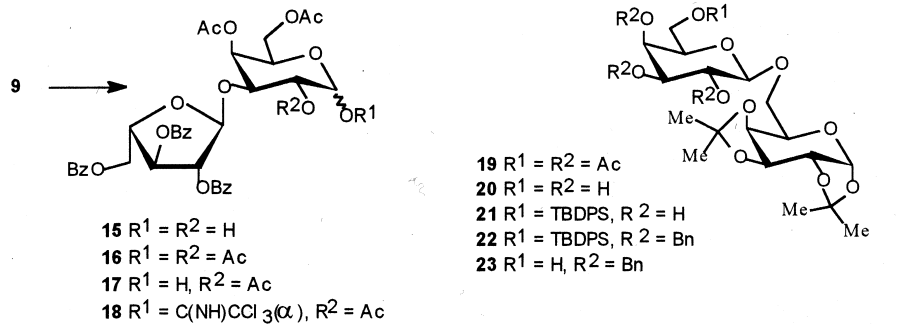
2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-(1→ 6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (24). A solution of **18** (190 mg, 0.21 mmol) and **23** (140 mg, 0.2 mmol) in anhydrous dichloromethane (5 mL) was cooled to 0°C, then to this solution was added TMSOTf (12 μL, 0.04 mmol) under nitrogen protection. The mixture was stirred at rt for 1 h, at the end of which time TLC (1/1 petroleum ether-ethyl acetate) showed that the reaction was complete. The reaction mixture was neutralized with triethylamine (0.01 mL), then concentrated. Column chromatography (1:1 petroleum ether-ethyl acetate) of the residue gave **24** as a syrup (233 mg, 81%). $[\alpha]_D^{25}$ −8° (*c* 3.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.13, 1.24, 1.26, 1.47 (4 s, 12 H, CH_3), 1.87, 2.02, 2.14 (3 s, 9 H, CH_3CO), 3.46–3.50 (m, 2 H, H-5^B), H-6^A), 3.61 (dd, 1 H, J 7.4, 9.3 Hz, H-6b^A), 3.72–3.73 (br d, 2 H, 2 H-6^B), 3.78–3.86 (m, 4 H, H-3^C, H-2^B, 2 H-6^C), 4.00–4.16 (m, 4 H, H-3^B, H-4^B, H-5^A), H-5^C), 4.18 (dd, 1 H, J 1.7, 8.0 Hz, H-3^A), 4.24 (dd, 1 H, J 5.0 Hz, H-2^A), 4.37 (d, 1 H, J 7.7Hz, H-1^B), 4.55 (dd, 1 H, J 2.4, 8.0 Hz, H-4^A), 4.58 (d, 1 H, J 8.1 Hz, H-1^C), 4.65 (d, 1 H, J 11.5 Hz, one proton of PhCH_2), 4.69–4.75 (m, 3 H, H-5^D, PhCH_2), 4.79–4.82 (m, 2 H, H-4^D and one proton of PhCH_2), 4.89–4.94 (m, 2 H, one proton of PhCH_2 and H-5^D), 5.04 (d, 1 H, J 11.0 Hz, one proton of PhCH_2), 5.25 (dd, 1 H, J 9.8, 8.1 Hz, H-2^C), 5.30 (s, 1 H, H-1^D), 5.36 (s, 1 H, H-2^D), 5.46 (d, 1 H, J 3.1 Hz, H-4^C), 5.50 (d, 1 H, J 5.0 Hz, H-1^A), 5.57 (d, 1 H, J 5.2 Hz, H-3^D), 7.26–8.10 (m, 30 H, Ph).

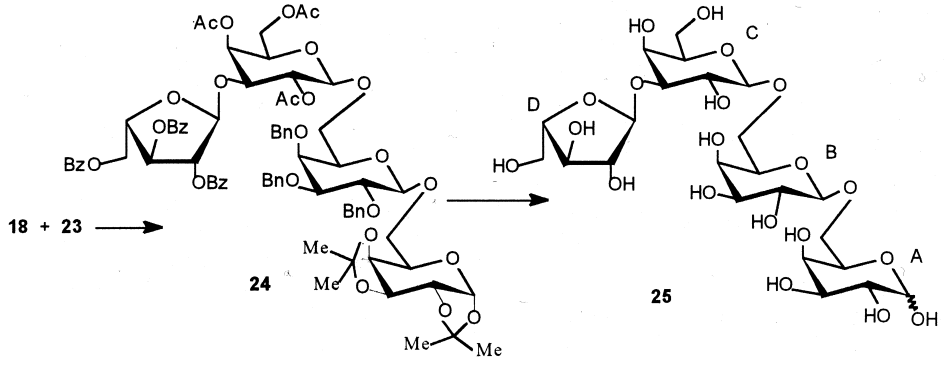
Anal. Calcd for $C_{77}H_{84}O_{26}$: C, 64.89; H, 5.90. Found: C, 64.78; H, 5.83.

α-L-Arabinofuranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-D-galactopyranose (25). A solution of **24** (198 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (2 mL) and the mixture was treated with 95% TFA (4 mL) for 2 h and then co-evaporated with toluene to dryness under reduced pressure. The syrup generated above was fully acetylated with acetic anhydride (1.5 mL) in pyridine (4 mL) for 5 h at rt, then co-evaporated with toluene (3 × 7 mL) to dryness. The resulting colorless oil was dissolved in MeOH/EtOAc/H_2O (3/1/0.05, v/v/v, 10 mL) and Pd/C (10%, 200 mg) was added. Hydrogen was bubbled into the mixture at rt for 24 h, during which time solvents were added occasionally and then filtered. The filtrate was concentrated and the residue was dissolved in MeOH (10 mL). NaOMe (0.5 M, keep pH at 9) was added and the









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mixture was stirred at room temperature overnight. After neutralization with Amberlite IR 120 (H^+), the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by Sephadex LH-20 column chromatography using MeOH as the eluent to afford **25** (43 mg, 49% from **24**). $[\alpha]_D^{25}$ −7° (*c* 0.3, H_2O); ^{13}C NMR (D_2O, 100 MHz): δ 63.0 (C-6^C), 63.6 (C-5^D), 69.8, 69.9, 71.1, 71.5, 71.8, 72.7, 73.5, 73.8, 73.9, 74.2, 74.6, 75.2, 76.9, 79.2, 80.5, 83.2, 85.9, 104.1, 105.5, 105.7 (C-1^A/C-1^B/C-1^C), 111.6 (C-1^D). ESIMS calcd for $C_{23}H_{40}O_{20}$ (636). Found ESI-MS(-): 635 $(M-H)^+$.

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