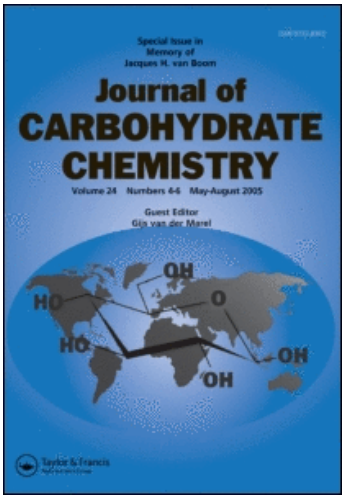


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# Synthesis of a Trisaccharide Repeating Unit Related to Arabinogalactan-Protein (AGP) Polysaccharides

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# SYNTHESIS OF A TRISACCHARIDE REPEATING UNIT RELATED TO ARABINOGALACTAN-PROTEIN (AGP) POLYSACCHARIDES[1]

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## ABSTRACT

Starting from L-arabinose and methyl β-D-galactopyranoside, methyl 2,3,4-tri-*O*-benzyl-6-*O*-[2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside **10** has been synthesized. Removal of protecting groups gave the methyl glycoside **12** of a trisaccharide representative of a repeating unit of arabinogalactan (AGP) polysaccharides.

## INTRODUCTION

Arabinogalactan-proteins (AGPs) are an important class of proteoglycans/glycoproteins widely distributed in plant tissues and exudates.[2] Their precise biological functions in plants remain unknown but, using a panel of monoclonal antibodies, it has been demonstrated that the presence of certain AGP epitopes are closely related to cell development in plant morphogenetic processes.[3,4,5] It is generally considered that the antibodies in question interact with epitopes arising from the predominant (~ 98%) carbohydrate component of the proteoglycans and have been shown to cross-react with the type of AGP typified by *Lolium multiforum* (ryegrass) arabinogalactan-protein. This polysaccharide is characteristic of type II arabinogalactans and has a linear β-(1-3)

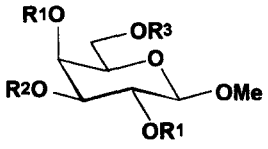
galactan backbone carrying short β-(1-6) galactan side branches substituted with arabinofuranose units.[6,7] More recently, oligosaccharide fragments from structurally similar arabinogalactans isolated from the Chinese herbs, *Angelica acutiloba* and *Bupleurum falcatum* have been shown to have various potential pharmacological activities.[8]

At present there is little information concerning the detailed composition and number of sugar units that constitute the carbohydrate epitopes in the antibody interactions and very few well-defined arabinose-containing oligosaccharides are available for biological studies. Accordingly, to provide model examples of these putative bioactive oligosaccharides, we have undertaken the synthesis of some AGP fragments of well-defined composition.[9,10] Initially these will be utilised to provide structural parameters that may be relevant to biological activity and will also be used in immunochemical studies as potential hapten inhibitors of anti-AGP monoclonal antibodies.



## RESULTS AND DISCUSSION

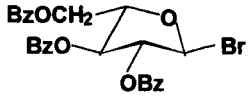
Synthesis of the target oligosaccharide has already been achieved in earlier studies employing a stepwise synthetic procedure.[10] We now report a more efficient synthesis of **12** using a block condensation approach employing a disaccharide glycosyl donor. This involved the synthesis of 2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-α-D-galactopyranosyl chloride **9** from the previously prepared methyl glycoside **8**. The galactopyranosyl acceptor **5**,[11] unsubstituted at the *O*-3 position, was synthesized from methyl β-D-galactopyranoside **1** in three steps. Compound **1** was regioselectively *p*-methoxybenzylated[12] (81% yield) *via* a dibutyl tin-oxide mediated reaction[13] with *p*-methoxybenzyl chloride as reagent and tetrabutylammonium iodide as catalyst to give **3**. The latter was then benzoylated and the free *O*-3 position was provided (88% yield) by oxidative removal of the methoxy substituted benzyl ether group with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in neutral conditions.[14] For the arabinofuranosyl donor, we utilised the 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide **2** readily obtained from L-arabinose.[15]



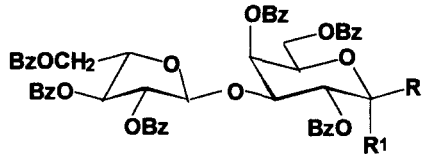

1 - 7

| | R1 | R2 | R3 |
|---|---|---|---|
| 1 | H | H | H |
| 3 | H | *p*-$CH_3OBn$ | H |
| 4 | Bz | *p*-$CH_3OBn$ | Bz |
| 5 | Bz | H | Bz |
| 6 | H | H | Tr |
| 7 | Bn | Bn | H |

Bz = benzoyl
Bn = benzyl

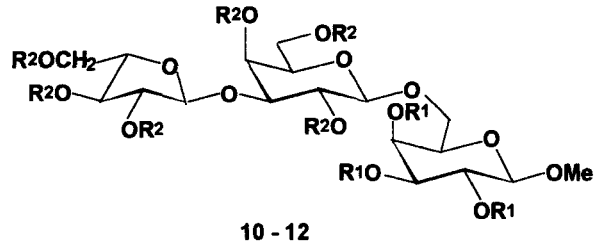



2




8 - 9

| | R | R1 |
|---|---|---|
| 8 | OMe | H |
| 9 | H | Cl |




10 - 12

| | R1 | R2 |
|---|---|---|
| 10 | Bn | Bz |
| 11 | Bz | Bz |
| 12 | H | H |

The coupling reaction between **2** and **5** was performed in dichloromethane in the presence of silver trifluoromethanesulfonate[16] as promoter and *sym*-collidine[17] as base yielding 85% of the α-L-linked disaccharide derivative **8**. The *trans* disposition of H-1 and H-2 of the L-arabinofuranosyl moiety was indicated by the characteristic coupling constant ($J_{1,2}$ <1 Hz) and confirmed[18] in $^{13}C$ NMR by the C-1 chemical shift (δ 107.90 ppm, see experimental). The high stereoselectivity of this glycosylation reaction is worth noting and no trace of 1,2-*cis* glycosidic linkage was detectable on TLC. As in previous work involving direct conversion of benzoylated methyl galactobiosides[19] to glycosyl chlorides, treatment of **8** with dichloromethyl methyl ether[20] (DCMME) gave the disaccharide glycosyl chloride **9** (68% yield) in the presence of zinc chloride as catalyst, the reaction conditions being such as to avoid cleavage of the arabinofurano glycosidic bond.

To provide the required protected trisaccharide **10**, in the second stage of coupling, the donor **9** was condensed with the methyl galactopyranosyl acceptor **7** unsubstituted at *O*-6 and benzylated at the remaining positions. The synthesis of **7**[11] involved the mono-*O*-tritylation[21] of **1** followed by successive *O*-benzylation and *O*-detritylation. Silver triflate catalysed the coupling of **9** with **7** in dichloromethane without base to afford the fully protected trisaccharide derivative **10** with an excellent yield of 80%. A shorter reaction time, but lower yield (45% not optimized), was achieved (see experimental) when the same donor **9** was coupled to the methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside[22] in the synthesis of the fully acylated trisaccharide **11** with silver triflate and *sym*-collidine. It has been noted previously[19,23] that, as in the present study, benzyl groups adjacent to the glycosylation position in the nucleophile result in higher formation of β-linked products.



Removal of the ester groups in **10** by Zemplén deacylation[24] furnished the corresponding debenzoylated compound. The target methyl 6-*O*-(3-*O*-α-L-arabinofuranosyl-β-D-galactopyranosyl)-β-D-galactopyranoside **12** was obtained after cleavage of benzyl groups by hydrogenolysis with palladium/charcoal as catalyst (overall deprotection 63% yield).

The $^{13}C$ and $^{1}H$ NMR assignments of **8**, **9**, **10**, **11** and **12** were made from $^{1}H$ - $^{1}H$ double quantum filter (DQF) COSY and $^{1}H$ - $^{13}C$ heteronuclear multiple quantum coherence (HMQC) NMR spectroscopy experiments.

## EXPERIMENTAL

**General methods.** Melting points were determined in capillary tubes with a Stuart melting-point apparatus and are uncorrected. Optical rotations were determined with a Thorn Type 243 automatic polarimeter at 25 °C using a 1-cm cell. Elemental analyses were performed by the analytical service of the School of Chemistry, University of Leeds. All reactions were monitored by thin-layer chromatography run on aluminium plates precoated with silica gel $60F_{254}$ (Merck, Darmstadt, Germany); detection was effected by charring with 5% sulphuric acid in ethanol and, when applicable, UV light (254 nm).

Column chromatography was performed using silica gel 60 (0.063-0.200 mm, Merck). Both chromatographic techniques were carried out with the following eluting solvents: A, dichloromethane/methanol; B, dichloromethane/ petroleum light; C, dichloromethane/ethyl acetate; D, ethyl acetate/methanol. For chromatography of the glycosyl chloride, the silica gel was dried at 160 °C overnight. Toluene and dichloromethane were dried with calcium hydride, distilled and stored over 3Å molecular sieves. DMF was dried at reflux temperature with calcium sulfate before being distilled under reduced pressure and stored in the dark over 3Å molecular sieves. Solutions in organic solvents were dried over sodium sulfate and concentrated under reduced pressure at ≤ 40 °C. All coupling reactions were carried out under argon. 1D and 2D NMR spectra were recorded with a Varian Unity 500 spectrometer operating at 500 MHz for $^{1}H$ and 125 MHz for $^{13}C$ using $CDCl_3$ (Aldrich, catalog No 15,183-1) and $D_2O$ (Aldrich, catalog No 34,377-3) as solvents with $Me_4Si$ (1% v/v) and 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (1% w/w) as internal standard respectively.



**Methyl 3-*O*-*p*-Methoxybenzyl-β-D-galactopyranoside (3).** A mixture of **1** (3.7 g, 19 mmol) and dibutyltin oxide (4.74 g, 19 mmol) in dry toluene (85 mL) was refluxed overnight. Tetrabutylammonium iodide (7 g, 19 mmol) was then added to the clear solution followed by *p*-methoxybenzyl chloride (3.36 mL, 24.8 mmol) and the reaction mixture was stirred for a further 1½ h at reflux temperature. Examination by TLC [solvent A (8:1)] showed that only traces of **1** remained and predominant presence of **3** ($R_F$ 0.45). The dark orange solution was concentrated to dryness and the resulting residue was chromatographed [solvent A (20:1) then (8:1)] to give amorphous **3** (4.83 g, 81%) after removal of several faster moving products and some traces of methyl 6-*O*-*p*-methoxybenzyl-β-D-galactopyranoside ($R_F$ 0.31). A portion of **3** was crystallized from methanol/diethyl ether to give pure sample: mp 128-130 °C; $[\alpha]_D$ +42° (*c* 1.0, MeOH); $^{1}H$ NMR ($D_2O$) δ 3.495-3.609 (m, 3H, H-2, H-3 and H-5), 3.560 (s, 3H, $OCH_3$), 3.723, 3.777 (2bd, 2H, H-6a and H-6b), 3.839 (s, 3H, *p*-$CH_3OBn$), 4.069 (bs, 1H, $J_{4,5} < 1$ Hz, H-4), 4.288 (d, 1H, $J_{1,2} = 7$ Hz, H-1), 4.570, 4.680 (2d, 2H, $J_{CH2,Ph} = 11$ Hz, $CH_2$ benzyl), 7.015 and 7.411 (2d, 4H, $H_{Ar}$); $^{13}C$ NMR ($D_2O$) δ 58.12 (*p*-$CH_3OBn$), 59.89 ($OCH_3$),

63.76 (C-6), 67.98 (C-4), 72.53 (C-2), 73.43 ($CH_2$ benzyl), 77.80 (C-5), 82.46 (C-3), 106.51 (C-1), 116.83 and 133.12 ($C_{Ar}$).

Anal. Calcd for $C_{15}H_{22}O_7$: C, 57.32; H, 7.05. Found: C, 57.60; H, 7.25.

**Methyl 2,4,6-Tri-*O*-benzoyl-3-*O*-*p*-methoxybenzyl-β-D-galactopyranoside (4).** A solution of **3** (2.46 g, 7.83 mmol) in pyridine (32 mL) was treated with benzoyl chloride (4.5 mL) at 0 °C and the mixture was stirred overnight at room temperature. The reaction was quenched by addition of ice-water and the organic layer was extracted with dichloromethane. The extract was washed with aqueous sodium hydrogen carbonate and water then dried and concentrated. Chromatography [solvent B (5:1) then solvent C (10:1)] of the residue yielded **4** (4.64 g, 94%) as a colourless foam: $[\alpha]_D$ +66° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.516 (s, 3H, $OCH_3$), 3.707 (s, 3H, *p*-$CH_3OBn$), 3.796 (dd, 1H, $J_{3,4}$ = 3 Hz, H-3), 4.091 (bt, 1H, $J_{5,6a}$ = 6.5 Hz, $J_{5,6b}$ = 6 Hz, H-5), 4.417-4.462 and 4.616-4.640 (2m, 4H, H-6a, H-6b and $CH_2$ benzyl), 4.536 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 5.504 (t, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 5.898 (d, 1H, H-4), 6.601, 7.043, 7.970, 8.054, 8.170 and 7.433-7.598 (5d and m, 19H, $H_{Ar}$); $^{13}C$ NMR ($CDCl_3$) δ 55.13 (*p*-$CH_3OBn$), 56.87 ($OCH_3$), 62.65 (C-6), 66.71 (C-4), 70.65 ($CH_2$ benzyl), 71.16 (C-2), 71.39 (C-5), 75.77 (C-3), 102.37 (C-1), 113.66, 128.23-133.38 ($C_{Ar}$).

Anal. Calcd for $C_{36}H_{34}O_{10}$: C, 69.00; H, 5.47. Found: C, 69.10; H, 5.20 .

**Methyl 2,4,6-Tri-*O*-benzoyl-β-D-galactopyranoside (5).**[11] To a stirred solution of **4** (1.73 g, 2.77 mmol) in dichloromethane (50 mL) containing water (2.5 mL, 1/20 of $CH_2Cl_2$) was added 2,3-dichloro-5,6-dicyanobenzoquinone (1.26 g, 5.54 mmol) at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred overnight at room temperature. After 24 h, when the reaction was shown to be complete [TLC, solvent C (30:1)], saturated aqueous sodium hydrogen carbonate was added and the mixture was extracted with dichloromethane. The organic extracts were washed with saturated aqueous sodium hydrogen carbonate, saturated aqueous sodium chloride, dried and concentrated. The residue was chromatographed [solvent C (30:1) then (10:1)] to yield **5** (1.23 g, 88%) as a colourless foam: $[\alpha]_D$ +6° (*c* 1.0, $CHCl_3$) {lit.[25] $[\alpha]_D$ +8.3° (*c* 1.5, $CHCl_3$)}.

**Methyl 6-*O*-Trityl-β-D-galactopyranoside (6).**[11] To a solution of **1** (970 mg, 5 mmol) in DMF (20 mL) containing trityl chloride (2.09 g, 7.5 mmol) and 4-dimethyl-

aminopyridine (61 mg, 0.5 mmol) was added triethylamine (1.4 mL, 10 mmol) and the mixture was stirred overnight at room temperature. After removal of DMF under reduced pressure, the coloured residue was partitioned between ice-water and dichloromethane. The organic extracts were washed with saturated aqueous ammonium chloride, water, dried and concentrated. The slightly yellow crystalline residue was readily recrystallized from ethanol to give compound **6** (1.67 g, 77%): mp 167-169 °C; $[\alpha]_D$ -48° (*c* 1.0, $CHCl_3$) {lit.[26] mp 184-185 °C ($CHCl_3$), $[\alpha]_D$ -38° (*c* 1.1, $CHCl_3$); lit.[28] mp 167-168 °C (EtOH)}.

**Methyl 2,3,4-Tri-*O*-benzyl-β-D-galactopyranoside (7).**[11] To a suspension of sodium hydride (135 mg, 4.5 mmol) in DMF (3 mL), a solution of **6** (436 mg, 1 mmol) in DMF (1.5 mL) was added dropwise at 0 °C. The mixture was stirred at 0-5 °C for 1 h and a solution of benzyl bromide (535 μL, 4.5 mmol) in DMF (0.5 mL) was then added dropwise. The mixture was allowed to warm up to room temperature and stirred for 10 h. Excess sodium hydride was destroyed with a few drops of methanol, and DMF was removed under reduced pressure. The residue was partitioned between dichloromethane and water. The organic layer was separated, washed with saturated aqueous sodium chloride, dried and concentrated. The resulting yellow syrup was *O*-detritylated as follows: acetyl chloride (500 μL) was added dropwise to methanol (10 mL) cooled in a cold water bath and to the fresh methanolic hydrogen chloride solution was added a solution of the above residue in diethyl ether (10 mL). The mixture was stirred at room temperature for 16 h [TLC, solvent C (5:1)]. Brine was added to the reaction mixture and after three extractions with diethyl ether, the combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate until neutral, then water, then dried and concentrated. Thus, compound **7** was obtained (258 mg, 56%) after purification by column chromatography [solvent C (10:1) then (5:1)] as a white foam: $[\alpha]_D$ -22° (*c* 1.0, $CHCl_3$) {lit.[27] $[\alpha]_D$ +42° (*c* 0.4, EtOH)}; $^1H$ NMR ($CDCl_3$) δ 3.384 (bt, 1H, $J_{5,6a}$ = 6.5 Hz, H-5), 3.490-3.544 (m, 2H, H-6a, H-6b), 3.557 (s, 3H, $OCH_3$), 3.765 (m, 1H, H-3), 3.784 (bd, 1H, $J_{3,4}$ = 2.5 Hz, H-4), 3.828 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 4.289 (d, 1H, $J_{1,2}$ = 8 Hz, 7.317-7.353 (m, 15H, $H_{Ar}$); $^{13}C$ NMR ($CDCl_3$) δ 56.97 ($OCH_3$), 61.80 (C-6), 73.00 (C-4), 73.34, 74.10 ($2CH_2$ benzyl), 74.52 (C-5), 75.14 ($CH_2$ benzyl), 79.60 (C-2), 82.10 (C-3), 104.98 (C-1), 127.48-128.39 ($C_{Ar}$).

**Methyl 2,4,6-Tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-galactopyranoside (8).** A solution of the bromide **2** (1.68 g, 3.2 mmol), the nucleophile **5** (1.01 g, 2 mmol), and *sym*-collidine (330 μL, 2.5 mmol) in dichloromethane (15 mL) was added dropwise to a suspension of silver triflate (1.02 g, 4 mmol) in dichloromethane (8 mL) at 0 °C under anhydrous conditions. At the end of the addition, and after 30 min at 0 °C, examination by TLC [solvent C (30:1)] showed that only traces of **5** remained and that one major product ($R_F$ 0.53) was formed. To isolate that product, the solution was made neutral with *sym*-collidine and the reaction mixture was diluted with dichloromethane. After filtration through a bed of Celite, the filtrate was washed with 5% aqueous sodium hydrogen carbonate, water, then dried and concentrated. Chromatography [solvent C (30:1) then (20:1)] of the residue yielded **8** (1.615 g, 85%) as a colourless foam: $[\alpha]_D$ +44° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.533 (s, 3H, $OCH_3$), 4.187 (t, 1H, $J_{5,6b}$ = 6.5 Hz, H-5), 4.275 (dd, 1H, $J_{3,4}$ = 3.5 Hz, H-3), 4.397 (dd, 1H, $J_{5,6a}$ = 6 Hz, $J_{6a,6b}$ = 11 Hz, H-6a), 4.565 (m, 1H, H-6b), 4.599 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.654 (dd, 1H, $J_{4',5'a}$ = 3.5 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5'a), 4.808 (bs, 1H, H-4'), 4.887 (dd, 1H, $J_{4',5'b}$ = 2.5 Hz, H-5'b), 5.269 (bs, 1H, H-2'), 5.370 (bs, $J_{1',2'}$ < 1 Hz, H-1'), 5.472 (bd, 1H, $J_{3',4'}$ = 5 Hz, H-3'), 5.729 (dd, 1H, $J_{2,3}$ = 10.2 Hz, H-2), 5.927 (bd, 1H, H-4), 7.718-8.036 (m, 30H, $H_{Ar}$); $^{13}C$ NMR ($CDCl_3$) δ 56.99 ($OCH_3$), 62.44 (C-6), 63.24 (C-5'), 69.95 (C-4), 71.54, 71.68 (C-2 and C-5), 76.68 (C-3), 77.50 (C-3'), 81.67 (C-4'), 82.53 (C-2'), 102.46 (C-1), 107.90 (C-1'), 128.13-133.29 ($C_{Ar}$).

Anal. Calcd for $C_{54}H_{46}O_{16}$: C, 68.20; H, 4.88. Found: C, 67.90; H, 4.95.

**2,4,6-Tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-α-D-galactopyranosyl chloride (9).** Disaccharide **8** (718 mg, 0.755 mmol) was dissolved in dry alcohol-free chloroform (7.5 mL) and dichloromethyl methyl ether (3.4 mL, 38 mmol) was added followed by zinc chloride (catalytic amount, ~25-30 mg). The mixture was stirred at 55 °C for 3 h when TLC [solvent C (40:1)] showed the formation of a major faster moving product ($R_F$ 0.60). After cooling, the reaction mixture was diluted with dichloromethane, filtered through a small bed of Celite and concentrated. The residue was chromatographed [solvent C (30:1)] to give **9** (490 mg, 68%) as a colourless foam: $[\alpha]_D$ +65° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 4.423 (dd, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 11.7 Hz, H-6a), 4.527 (dd, 1H, $J_{5,6b}$ = 6.5 Hz, H-6b), 4.737 (m, 2H, H-3 and H-5'a), 4.826 (m, 1H, H-

5), 4.895 (bs, 1H, H-4'), 4.981 (dd, 1H, $J_{4',5'b}$ = 2.5 Hz, $J_{5'a,5'b}$ = 12.2 Hz, H-5'b), 5.367 (bs, 1H, H-2'), 5.556 (bd, 1H, $J_{3',4'}$ = 4 Hz, H-3'), 5.585 (bs, 1H, H-1'), 5.756 (dd, 1H, $J_{2,3}$ = 10 Hz, H-2), 6.082 (bs, 1H, H-4), 6.662 (d, 1H, $J_{1,2}$ = 4 Hz, H-1), 7.183-8.093 (m, 30H, $H_{Ar}$); $^{13}$C NMR ($CDCl_3$) δ 62.19 (C-6), 63.14 (C-5'), 70.18 (C-4), 70.61 (2C, C-2 and C-5), 72.26 (C-3), 77.60 (C-3'), 81.62 (C-4'), 82.45 (C-2'), 91.97 (C-1), 107.92 (C-1'), 128.29-133.38 ($C_{Ar}$).

Anal. Calcd for $C_{53}H_{43}ClO_{15}$: C, 66.63; H, 4.54. Found: C, 66.20; H, 4.20.

**Methyl 2,3,4-Tri-*O*-benzyl-6-*O*-[2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside (10).** To a stirred solution of the nucleophile **7** (106 mg, 0.228 mmol), silver triflate (117 mg, 0.456 mmol) and powdered 4Å molecular sieves (110 mg) in dichloromethane (1 mL) was added, dropwise at room temperature, the disaccharide glycosyl chloride **9** (300 mg, 0.314 mmol) in dichloromethane (2 mL). After 24 h in the dark, examination by TLC [solvent C (24:1)] showed that only traces of **7** remained and that one major product ($R_F$ 0.48) was formed. The reaction mixture was diluted with dichloromethane and filtered through a bed of Celite. The filtrate was washed with 5% aqueous sodium hydrogen carbonate, water, then dried and concentrated. The residue was chromatographed [solvent C (22:1) then (16:1)] yielding **10** (254 mg, 80%) as a colourless foam: $[\alpha]_D$ +28° (*c* 1.0, $CHCl_3$); $^1$H NMR ($CDCl_3$) δ 3.250 (s, 3H, $OCH_3$), 3.357 (m, 1H, H-3), 3.464 (bt, 1H, $J_{5,6a}$ = 6 Hz, H-5), 3.746 (m, 3H, H-2, H-4 and H-6a), 3.933 (dd, 1H, $J_{5,6b}$ = 5 Hz, $J_{6a,6b}$ = 9.7 Hz, H-6b), 4.095 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.180 (bt, 1H, $J_{5',6'a}$ = 6 Hz, H-5'), 4.272 (m, 1H, H-3'), 4.370 (dd, 1H, $J_{6'a,6'b}$ = 11 Hz, H-6'a), 4.501-4.910 and 4.753 (m and d, 11H, $J_{1',2'}$ = 8 Hz, H-6'b, H-4", H-5"a, H-5"b, 3$CH_2$ benzyl and H-1'), 5.277 (bs, 1H, H-2"), 5.371 (bs, 1H, H-1"), 5.502 (bd, 1H, H-3"), 5.734 (m, 1H, H-2'), 5.953 (bd, 1H, H-4'), 7.187-8.036 (m, 45H, $H_{Ar}$); $^{13}$C NMR ($CDCl_3$) δ 56.64 ($OCH_3$), 62.32 (C-6'), 63.22 (C-5"), 68.75 (C-6), 69.97 (C-4'), 71.67, 71.74 (C-2' and C-5'), 72.93 ($CH_2$ benzyl), 73.58 (2C, C-2 and C-5), 74.48, 74.99 (2$CH_2$ benzyl), 76.57 (C-3'), 77.49 (C-3"), 79.34 (C-4), 81.57 (C-4"), 81.94 (C-3), 82.59 (C-2"), 101.70 (C-1'), 104.74 (C-1), 107.92 (C-1"), 127.49-133.24 ($C_{Ar}$).

Anal. Calcd for $C_{81}H_{74}O_{21}$: C, 70.32; H, 5.39. Found: C, 69.90; H, 5.10.

**Methyl 2,3,4-Tri-*O*-benzoyl-6-*O*-[2,4,6-tri-*O*-benzoyl-3-*O*-(2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl)-β-D-galactopyranosyl]-β-D-galactopyranoside (11).** A solution

of the chloride **9** (190 mg, 0.199 mmol), methyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside[22] (63 mg, 0.124 mmol) and *sym*-collidine (20.5 μL, 0155 mmol) in dichloromethane (2 mL) was added dropwise to a suspension of silver triflate (64 mg, 0.248 mmol) in dichloromethane (0.5 mL) at room temperature under anhydrous conditions. After 1¼ h at room temperature the solution was made neutral with *sym*-collidine. To isolate the desired product [solvent C (25:1) $R_F$ 0.46], the reaction mixture was diluted with dichloromethane, filtered through a bed of Celite and the filtrate was washed with 5% aqueous sodium hydrogen carbonate, water, and dried. The residue was chromatographed [solvent C (25:1)] to yield **11** (79 mg, 45%) as a glassy solid: $[\alpha]_D$ +70° (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 3.190 (s, 3H, $OCH_3$), 3.817 (dd, 1H, $J_{5,6a}$ = 8 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 4.148 (m, 3H, H-5, H-6b and H-5'), 4.249 (dd, 1H, $J_{3',4'}$ = 3.5 Hz, H-3'), 4.317 (dd, 1H, $J_{5',6'a}$ = 6 Hz, $J_{6'a,6'b}$ = 11 Hz, H-6'a), 4.392 (dd, 1H, $J_{5',6'b}$ = 7 Hz, H-6'b), 4.542 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.657 (m, 1H, H-5"a), 4.758 (d, 1H, $J_{1',2'}$ = 8 Hz, H-1'), 4.821 (bs, 1H, H-4"), 4.885 (m, 1H, H-5"b), 5.275 (bs, 1H, H-2"), 5.360 (bs, 1H, H-1"), 5.495 (m, 2H, H-3 and H-3"), 5.667 (dd, 1H, $J_{2,3}$ = 10 Hz, H-2), 5.743 (dd, 1H, $J_{2',3'}$ = 10 Hz, H-2'), 5.849 (bd, 1H, H-4), 5.900 (bd, 1H, H-4'), 7.189-8.035 (m, 45H, $H_{Ar}$); $^{13}C$ NMR ($CDCl_3$) δ 56.80 ($OCH_3$), 62.21 (C-6'), 63.20 (C-5"), 68.27 (C-6), 68.82 (C-4), 69.84 (2C, C-2 and C-4'), 71.48 (C-2'), 71.70 (2C, C-3 and C-5'), 73.23 (C-5), 76.51 (C-3'), 77.46 (C-3"), 81.60 (C-4"), 82.57 (C-2"), 101.43 (C-1'), 102.14 (C-1), 107.90 (C-1"), 128.12-133.43 ($C_{Ar}$).

Anal. Calcd for $C_{81}H_{68}O_{24}$: C, 68.25; H,4.81. Found: C, 67.85; H, 4.70.

**Methyl 6-*O*-(3-*O*-α-L-Arabinofuranosyl-β-D-galactopyranosyl)-β-D-galactopyranoside (12).** A solution of **10** (215 mg, 0.155 mmol) in 2:1 (v/v) methanol-toluene (6 mL) was treated with a catalytic amount of freshly prepared sodium methoxide in methanol (3 mL) at room temperature for 3 h. The reaction mixture was neutralized with 'Dowex' 50W-X8($H^+$) resin, filtered and concentrated to dryness. Column chromatography [solvent D (10:1) then (2.5:1)] of the residue gave the expected debenzoylated methyl glycoside (93 mg, 79%). A mixture of the latter (0.122 mmol) and 10% Pd/C (50 mg) in methanol (6 mL) was stirred at room temperature under hydrogen until the reaction was complete. The reaction mixture was filtered, washed with water and concentrated. The residue was purified by short column chromatography [solvent A (1:1.5)] to afford **12** (48

mg, 80%) as a white solid: $[\alpha]_D$ -57° (*c* 0.9, $H_2O$); $^1H$ NMR ($D_2O$) δ 3.499 (bt, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 3.570 (s, 3H, $OCH_3$), 3.619-3.828 (m, 8H, H-3, H-2', H-3', H-5', H-6'a, H-6'b, H-5"a and H-5"b), 3.926 (m, 4H, H-4, H-5, H-6a and H-3"), 4.075(m, 3H, H-6b, H-4' and H-4"), 4.197 (bs, 1H, H-2"), 4.332 (d, 1H, $J_{1,2}$ = 8 Hz, H-1), 4.514 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 5..220 (bs, 1H, H-1"); $^{13}C$ NMR ($D_2O$) δ 60.06 ($OCH_3$), 63.64 (C-6'), 63.94 (C-5"), 71.17, 71.40 (C-4 and C-4'), 71.66 (C-6), 72.59 (C-2'), 73.34 (C-2), 75.32 (C-3), 76.49 (C-5), 77.69 (C-5'), 79.23 (C-3"), 82.97 (C-3'), 83.99 (C-2"), 86.56 (C-4"), 105.74 (C-1'), 106.51 (C-1), 111.93 (C-1").

Anal. Calcd for $C_{18}H_{32}O_{15}.3H_2O$: C, 39.84; H, 7.06. Found: C, 39.45; H, 6.55.

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