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Carbohydrate Research 323 (2000) 28–35

CARBOHYDRATE RESEARCH

Synthesis of a tetrasaccharide representing a minimal epitope of an arabinogalactan

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Received 6 July 1999; accepted 6 September 1999

Abstract

The hydrophobic alkyl chain-containing tetrasaccharide, dodecyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→2)]-β-D-galactopyranoside, was synthesized efficiently using a convergent strategy. In coupling reactions, protected trichloroacetimidates proved to be better donors than their corresponding bromides in the preparation of the dodecyl disaccharide and trisaccharide. Zemplén deacylation provided the target tetramer in good overall yield.

Keywords: Carbohydrate; Arabinogalactan; Regioselective reaction; Antigen

1. Introduction

Arabinogalactan-proteins (AGPs) are an important class of proteoglycans/glycoproteins widely distributed in plant tissues and exudates. 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 is closely related to cell development in plant morphogenetic processes [1,2]. Arabinogalactan AG-II, extracted from Chinese herbs, the roots of *Angelica acutiloba* and *Bupleurum falcatum*, is a well-known component of Chinese herbal medicine for the treatment of gynecological diseases and arthritis [3].

There is no unambiguous report concerning the detailed composition of the arabinogalactan [4]. Albersheim and co-workers [5] demonstrated that the immunogenic region in this type of cell-wall polysaccharide consists of a β-(1→6)-linked galactan containing at least three D-galactopyranosyl residues functionalized at one hydroxyl group with an α-linked L-arabinofuranosyl unit. Although the structure and activity studies of AGIIb-1 show the importance of arabinofuranose side-chains in potential pharmacological activities [3], we do not yet have complete structural knowledge of these AGPs. Besides, it is very difficult to obtain arabinose-containing oligosaccharides of known structure for biological studies from natural sources. We present here the synthesis of a well-defined dodecylated arabinogalactan tetrasaccharide for use in antibody screening, immunogen development and inhibitor construction [6].

2. Results and discussion

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-1,2:3,4-di-*O*-isopropylidene-α-D-

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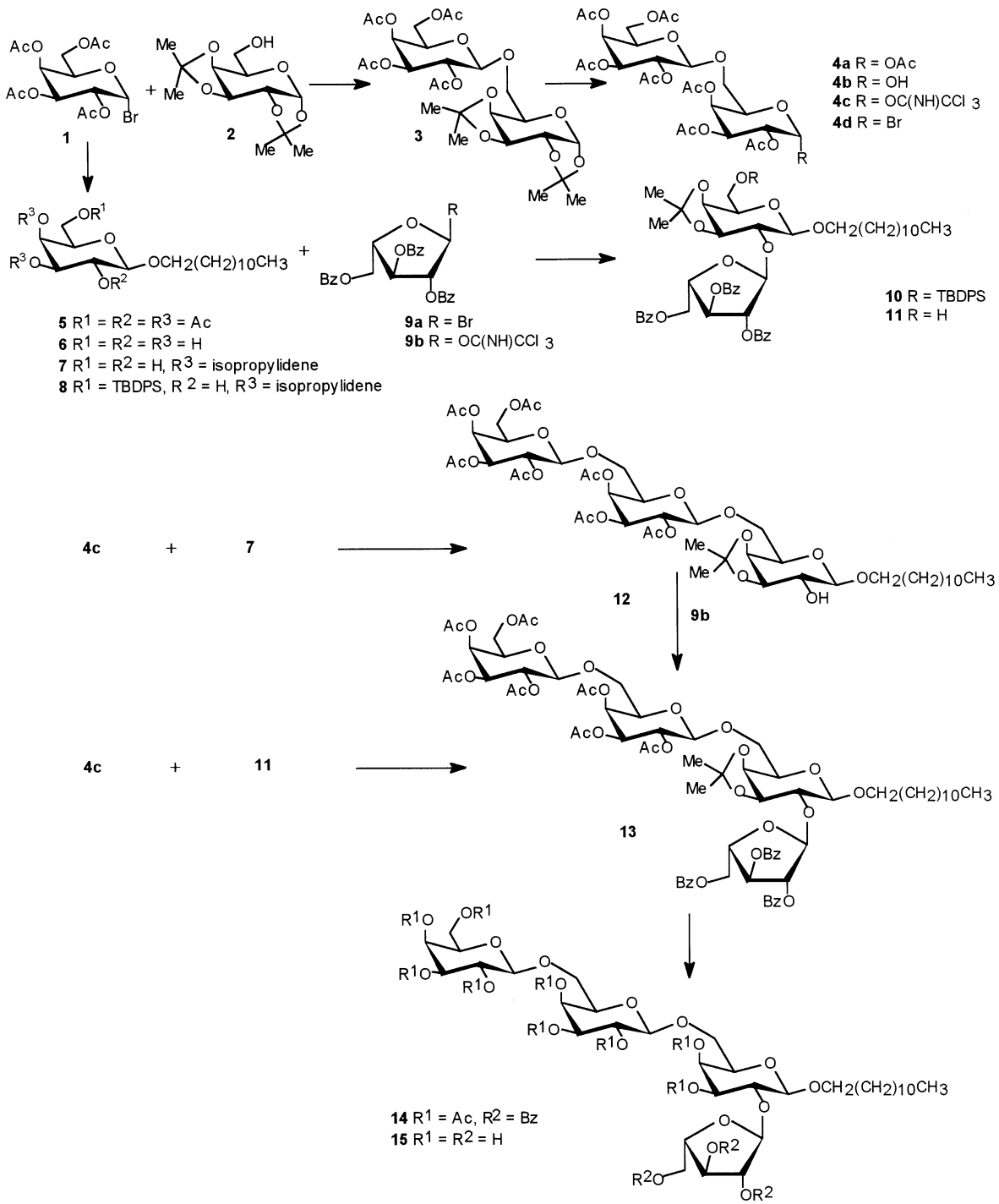
0008-6215/00/$ - see front matter
PII: S0008-6215(99)00252-9

galactopyranose (**3**) was prepared from 2,3,-4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**1**) and 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) using a modified Koenigs–Knorr reaction [7] (see Scheme 1). Compound **3** was deacetalated with 90% trifluroacetic acid (TFA), followed by acetylation with acetic anhydride in pyridine (→**4a**). Regioselective deacetylation at the anomeric carbon with benzylamine (→**4b**) [8], and activation with trichloroacetonitrile using 1,8-diazabicyclo[5.4.0]undec-7-ene(1,5-5) (DBU) as a catalyst, furnished the disaccharide donor 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl 2,2,2-trichloroacetimidate (**4c**). At the



Scheme 1.

same time, dodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**5**) was prepared by the classic Koenigs–Knorr reaction of **1** and dodecyl alcohol with promotion by silver oxide [9]. Zemplén deacetylation of **5** (→**6**), followed by regioselective O-isopropylidenation with 2,2-dimethoxypropane and *p*-toluenesulfonic acid, gave dodecyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**7**) in good yield (80% from **5**). Selective protection of the primary alcohol of **7** with *tert*-butylchlorodiphenylsilane in triethylamine gave dodecyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**8**). It is worth noting that the glycosylation between acceptor **8** and 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl bromide (**9a**) [10] with silver trifluoromethanesulfonate as the promotor was not satisfactory due to migration of dodecyl group from acceptor **8** to donor **9a**. Dodecyl 2,3,5-tri-*O*-benzoyl-L-arabinofuranoside was produced as a mixture in about 40% yield with the α isomer predominant. Alkyl migration did not appear to occur when octyl glucopyranoside was glycosylated with tetra-*O*-acetyl-α-D-glucopyranosyl bromide under the same reaction conditions [11]. Since **9a** did not work well in its coupling with **8**, it was converted to 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl 2,2,2-trichloroacetimidate (**9b**) by debromination with silver carbonate in 5:1 acetone–water, followed by Schmidt activation [12] in a total yield of 79% (two steps).

An attempt at regioselective glycosylation of **7** with **4d** as the donor using the orthoester formation–rearrangement method [13] was also disappointing due to dodecyl migration and the poor regioselectivity between 6-OH and 2-OH of the galactose moiety. While regioselective glycosylation of **7** with **4c** as the donor in CH_2Cl_2 at −42 °C using Me_3SiOTf as the catalyst gave predominantly dodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-3,4-*O*-isopropylidene-β-D-galactopyranoside (**12**, 59%), together with its 2-linked regioisomer, dodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-galactopyranoside (31%). Further coupling of **12** with **9b** furnished tetrasaccharide dodecyl 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→2)]-3,4-*O*-iso-propylidene-β-D-galactopyranoside (**13**) in 74% yield.

In order to improve the efficiency of the synthesis, a convergent strategy was used. Thus, acceptor **8** was first glycosylated with **9b** in the presence of Me_3SiOTf, affording dodecyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→2)-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**10**), which was desilylated with tetrabutylammonium fluoride (TBAF) in THF furnishing dodecyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→2)-3,4-*O*-isopropylidene-β-D-galactopyranoside (**11**). Coupling of the disaccharide donor **4c** with the acceptor **11** in the presence of Me_3SiOTf in anhydrous CH_2Cl_2 generated tetrasaccharide **13** in high yield (89%). Compound **13** was treated with 90% TFA, then acetylated with acetic anhydride in pyridine (→**14**) and fully deprotected by Zemplén deacylation, giving the target compound dodecyl β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-[α-L-arabinofuranosyl-(1→2)]-β-D-galactopyranoside (**15**) in a total yield of 70% (from **13**).

3. Experimental

General methods.—Optical rotations were determined at 25 °C with a Perkin–Elmer model 241MC automatic polarimeter. Melting points were determined with a 'Mel-Temp' apparatus. ^1H NMR, ^{13}C NMR and ^1H–^1H, ^1H–^{13}C COSY spectra were recorded with Bruker ARX 400 spectrometers for solutions in $CDCl_3$, MeOD 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 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 concd at < 60 °C under diminished pressure.

Preparation of 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→6)-1,2:3,4-di*-O-*isopropylidene-α*-D-*galactopyranose* (**3**).—To a soln of **1** (4.5 g, 11 mmol) and **2** (2.4 g, 10 mmol) in anhyd CH_2Cl_2 (80 mL) was added silver triflate (2.9 g, 11 mmol) in the presence of 4 Å molecular sieves under a N_2 atmosphere at −15 °C. The mixture was stirred in a dark room at room temperature (rt) for 2 h, neutralized with Et_3N and filtered, and then the filtrate was concd. Purification of the product by column chromatography (3:1 petroleum ether–EtOAc) gave crystalline **3** (4.86 g, 81%); mp 97–99 °C. [lit. [14]: 101–102 °C]; $[\alpha]_D^{25}$ −45° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 6 H, 2 CH_3), 1.45, 1.51 (2 s, 6 H, 2 CH_3), 1.98, 2.05, 2.09, 2.14 (4 s, 12 H, 4 CH_3CO), 3.68 (dd, 1 H, $J_{5,6a}$ 7.6, $J_{6a,6b}$ 11.4 Hz, H-6a), 3.90–3.95 (m, 2 H, H-5, H-6′a), 4.04 (dd, 1 H, $J_{5,6b}$ 3.2 Hz, H-6b), 4.11–4.19 (m, 3 H, H-3, H-5′, H-6′b), 4.29 (dd, 1 H, $J_{1,2}$ 5.0, $J_{2,3}$ 2.4 Hz, H-2), 4.58 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.59 (dd, 1 H, $J_{3,4}$ 8.0, $J_{4,5}$ 2.1 Hz, H-4), 5.02 (dd, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.4 Hz, H-3′), 5.22 (dd, 1 H, H-2′), 5.39 (dd, 1 H, $J_{4',5'}$ 0.7 Hz, H-4′), 5.00 (d, 1 H, H-1). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.0, 20.1, 20.1, 20.3, (4 *C*H_3), 24.0, 24.7, 25.6, 25.7 (4 *C*H_3CO), 60.9, 66.9, 67.5, 68.5, 69.1, 70.3, 70.4, 70.5, 70.6, 71.0 (C-2,3,4,5,6/C-2′,3′,4′,5′.6′), 95.9 (C-1), 101.7 (C-1′), 108.3 ((CH$_3$)$_2$*C*(OR)$_2$), 109.1 ((CH$_3$)$_2$*C*(OR)$_2$), 169.5, 169.7, 169.7, 169.8(4 *C*O).

2,3,4,6-Tetra-O-*acetyl-β*-D-*galactopyranosyl-(1→6)-2,3,4-tri*-O-*acetyl-α*-D-*galactopyranosyl 2,2,2-trichloroacetimidate* (**4c**).—Compound **3** (2.2 g, 3.7 mmol) was dissolved in aq 90% trifluoroacetic acid (10 mL). The soln was stirred at rt for 3 h and then co-evaporated with toluene to dryness under diminished pressure. The syrup thus generated was fully acetylated with Ac_2O (5 mL) in pyridine (8 mL) for 4 h at rt, then co-evaporated with toluene (2 × 20 mL) to dryness. Column chromatography using 2:1 petroleum ether–EtOAc as the eluent gave syrupy **4a** (2.0 g, 79%). To a soln of **4a** (2.10 g, 3.1 mmol) in THF (20 mL) was added benzylamine (3.4 mL, 31 mmol) under cooling with an ice-water bath. The soln was stirred at rt for 10 h, then 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 concd. Column chromatography (1:1 petroleum ether–EtOAc) of the residue gave **4b** as a syrup (1.6 g, 85%). To a soln of **4b** (2.1 g, 3.3 mmol) in anhyd CH_2Cl_2 (40 mL) was added trichloroacetonitrile (3.0 mL, 30 mmol) and DBU (0.45 mL, 3.0 mmol), and the mixture was stirred at rt for 2 h. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concd and purified on a silica gel column using 2:1 petroleum ether–EtOAc as eluent to give **4c** as a syrup (2.06 g, 80%); $[\alpha]_D^{25}$ −34° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 1.97, 2.01, 2.03, 2.03, 2.06, 2.15, 2.17 (7 s, 21 H, 7 CH_3CO), 3.71 (dd, 1 H, $J_{5,6a}$ 6.9, $J_{6a,6b}$ 10.9 Hz, H-6a), 3.80 (dd, 1 H, $J_{5,6b}$ 5.2 Hz, H-6b), 3.87–3.90 (m, 1 H, H-5). 4.08–4.17 (m, 2 H, H-6′a, H-6′b), 4.37–4.40 (m, 1 H, H-5′), 4.50 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.97 (dd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.4 Hz, H-2), 5.13 (dd, 1 H, $J_{2',3'}$ 10.4 Hz, H-2′), 5.34 (dd, 1 H, $J_{3,4}$ 3.5 Hz, H-4), 5.37 (dd, 1 H, $J_{4,5}$ 0.8 Hz, H-4), 5.41 (dd, 1 H, $J_{3',4'}$ 3.1 Hz, H-3′), 5.54 (dd, 1 H, $J_{4',5'}$ 1.1 Hz, H-4′), 6.58 (d, 1 H, H-1), 8.65 (s,1 H, N*H*). Anal. Calcd for $C_{28}H_{36}Cl_3NO_{18}$: C, 43.05; H, 4.61. Found: C, 43.11; H, 4.66.

Dodecyl 2,3,4,6-tetra-O-*acetyl-β*-D-*galactopyranoside* (**5**).—To a soln of compound **1** (9.4 g, 22.9 mmol) and dodecyl alcohol (6 g, 32.3 mmol) in anhyd ether was added silver oxide (9 g, 38.8 mmol) and 4 Å molecular sieves. The mixture was stirred in a dark room at rt for 16 h, then filtered, and the filtrate was concd to a syrup which was purified by column chromatography using 3:1 petroleum ether–EtOAc as the eluent, to give syrupy **5** (8.26 g, 70%); $[\alpha]_D^{25}$ −14° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.7 Hz, CH_3), 1.26 (bs, 18 H, 9 CH_2), 1.56–1.57 (m, 2 H, CH_2), 1.99, 2.04, 2.05, 2.15 (4 s, 12 H, CH_3CO), 3.44–3.50 (m, 1 H, one proton of –OCH_2(CH$_2$)$_{10}$CH$_3$), 3.86–3.92 (m, 2 H, H-5 and one proton of –OCH_2(CH$_2$)$_{10}$CH$_3$), 4.10–4.21 (m, 2 H, $J_{5,6a}$ 7.9, $J_{5,6b}$ 7.0, $J_{6a,6b}$ 11.2 Hz, H-6a, H-6b), 4.45 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1),

5.02 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.4 Hz, H-3), 5.21 (dd, 1 H, H-2), 5.39 (d, 1 H, H-4). Anal. Calcd for $C_{26}H_{44}O_{10}$: C, 60.46; H, 8.53. Found: C, 60.50; H, 8.51.

*Dodecyl 3,4-*O*-isopropylidene-β-*D*-galactopyranoside* (**7**).—A soln of **5** (3.2 g, 6.2 mmol) in MeOH (25 mL) was treated with NaOMe (3.0 mL, 0.5 M in MeOH) at rt for 6 h. The soln was neutralized with Dowex-50W (H^+) resin, then filtered, and the filtrate was evaporated to give **6** as a syrup. Compound **6** (1.7 g, 4.9 mmol) was dissolved in acetone (10 mL), and 2,2-dimethoxypropane (3.0 mL, 25 mmol) and *p*-toluenesulfonic acid monohydrate (25 mg) was added. The mixture was stirred at rt for 16 h, then neutralized with Et_3N (2.0 mL) and concd. Column chromatography (3:2 petroleum ether–EtOAc) of the residue gave **7** as a syrup (3.4 g, 80%). Compound **7** was converted into its acetylated derivative, dodecyl 2,6-di-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside showing the following physical data: $[\alpha]_D^{25} + 110°$ (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.7 Hz, C*H*$_3$), 1.25 (bs, 18 H, 9 C*H*$_2$), 1.34 (s, 3 H, C*H*$_3$), 1.55 (bs, 5 H, C*H*$_3$ and C*H*$_2$), 2.09, 2.13 (2 s, 6 H, 2 C*H*$_3$CO), 3.40–3.46 (m, 1 H, one proton of OC*H*$_2$(CH$_2$)$_{10}$CH$_3$), 3.83–3.88 (m, 1 H, one proton of OC*H*$_2$(CH$_2$)$_{10}$CH$_3$), 3.98 (dt, 1 H, $J_{4,5}$ 1.8, $J_{5,6}$ 6.1 Hz, H-5), 4.15–4.20 (m, 2 H, H-3 and H-4), 4.31 (d, 1 H, $J_{1,2}$ 8.2 Hz, H-1), 4.36 (d, 2 H, 2 H-6), 4.96 (dd, 1 H, $J_{2,3}$ 6.9 Hz, H-2). Anal. Calcd for $C_{25}H_{44}O_8$: C, 63.56; H, 9.32. Found: C, 63.55; H, 9.32.

*Dodecyl 3,4-di-*O*-isopropylidene-6-*O*-tert-butyldiphenylsilyl-β-D-galactopyranoside* (**8**).—Compound **7** (2.0 g, 5.15 mmol) was dissolved in CH_2Cl_2 (10 mL) and *tert*-butyldiphenylsilyl chloride (1.7 g, 6.18 mmol), Et_3N (10 mL) and 4-dimethylaminopyridine (30 mg) were added to the soln. The mixture was stirred at rt for 16 h, then poured into cold water, extracted with CH_2Cl_2 (2 × 30 mL), and the organic layer was dried over Na_2SO_4 and concd. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave **8** as a syrup (2.94 g, 91%); $[\alpha]_D^{25} + 1°$ (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, C*H*$_3$), 1.06 (s, 9 H, *t*-Bu), 1.21–1.31 (bs, 18 H, 9 C*H*$_2$), 1.35, 1.51 (2 s, 6 H, 2 C*H*$_3$), 1.56–1.62 (m, 2 H, C*H*$_2$), 2.22–2.41 (bs, 1 H, O*H*), 3.43–3.49 (m, 1 H, one proton of OC*H*$_2$R), 3.54 (t, 1 H, $J_{1,2} = J_{2,3} = 7.8$ Hz, H-2), 3.84–3.90 (m, 2 H, H-5 and one proton of OC*H*$_2$R), 3.93 (dd, 1 H, $J_{5,6a}$ 6.1, $J_{6a,6b}$ 9.8 Hz, H-6a), 3.99 (dd, 1 H, $J_{5,6b}$ 7.3 Hz, H-6b), 4.07 (dd, 1 H, $J_{3,4}$ 5.48 Hz, H-3), 4.14 (d, 1 H, H-1), 4.27 (dd, 1 H, $J_{4,5}$ 2.1 Hz, H-4), 7.37–7.72 (m, 10 H, 2 Ph). Anal. Calcd for $C_{37}H_{58}O_6Si$: C, 70.93; H, 9.26. Found: C, 70.95; H, 9.27.

*2,3,5-Tri-*O*-benzoyl-α-L-arabinofuranosyl 2,2,2-trichloroacetimidate* (**9b**).—To a soln of **9a** (800 mg, 1.64 mmol) in 1:1 acetone–water (10 mL) was added silver carbonate (905 mg, 3.28 mmol). The reaction mixture was stirred in a dark room at rt for 16 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was filtered, and the filtrate was concd to dryness. The syrup thus obtained was subjected to Schmidt activation with trichloroacetonitrile (0.3 mL, 3.0 mmol) and anhyd K_2CO_3 (1.09 g) in CH_2Cl_2 (5 mL) for 8 h at rt. The mixture was filtered, and the filtrate was concd. Purification of the residue on column chromatography (3:1 petroleum ether–EtOAc) gave **9b** as a glassy solid (785 mg, 79%); $[\alpha]_D^{25} - 24°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.73–4.86 (m, 3 H, H-4,5a,5b), 5.68 (d, 1 H, $J_{3,4}$ 3.3 Hz, H-3), 5.81 (s, 1 H, H-2), 6.67 (s, 1 H, H-1), 7.24–8.13 (m, 15 H, 3 PhCO), 8.73 (s, 1 H, N*H*). Anal. Calcd for $C_{28}H_{22}Cl_3NO_8$: C, 55.42; H, 3.66. Found: C, 55.13; H, 3.99.

*Dodecyl 2,3,5-tri-*O*-benzoyl-α-L-arabinofuranosyl-(1→2)-6-*O*-tert-butyldiphenylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside* (**10**).—To a mixture of **8** (760 mg, 1.19 mmol) and **9b** (788 mg, 1.3 mmol), 4 Å molecular sieves, and anhyd CH_2Cl_2 (15 mL) was added Me_3SiOTf (20 μL, 0.11 mmol) under a N_2 atmosphere at −15 °C. The mixture was stirred under these conditions for 1 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that all starting material **9b** was consumed. The reaction mixture was neutralized with Et_3N, then filtered, and the filtrate was concd. Purification of the product by column chromatography (3:1 petroleum ether–EtOAc) gave **10** as a syrup (1.13 g, 87%); $[\alpha]_D^{25} + 82°$ (*c* 1.0, $CHCl_3$); ^{1}H NMR

($CDCl_3$): δ 0.87 (t, 3 H, CH_3), 1.06 (bs, 25 H, 8 CH_2 and (CH_3)$_3$C), 1.08–1.25 (m, 4 H, 2 CH_2), 1.29, 1.55 (2 s, 6 H, 2 CH_3), 3.29–3.35 (m, 1 H one proton of OCH_2R), 3.77–3.81 (m, 1 H, one proton of OCH_2R), 3.83–3.90 (m, 2 H, H-2, H-5), 3.92–4.01 (m, 2 H, $J_{5,6a}$ 6.2, $J_{5,6b}$ 7.4, $J_{6a,6b}$ 9.9 Hz, H-6a, H-6b), 4.25–4.30 (m, 3 H, $J_{1,2}$ 8.5 Hz, H-1, H-3, H-4), 4.71 (dd, 1 H, $J_{4',5'a}$ 5.5, $J_{5'a,5'b}$ 12.3 Hz, H-5′a), 4.77–7.82 (m, 2 H, $J_{4',5'b}$ 3.0 Hz, H-4′, H–5′b), 5.54 (d, 1 H, $J_{3',4'}$ 4.5 Hz, H-3′), 5.64 (s, 1 H, H-1′/H-2′), 5.72 (s, 1 H, H-2′/H-1′), 7.23–8.13 (m, 25 H, 5 Ph). Anal. Calcd for $C_{63}H_{78}O_{13}Si$: C, 70.65; H, 7.29. Found: C, 70.71; H, 7.23.

Dodecyl 2,3,5-tri-O-*benzoyl*-α-L-*arabino*-*furanosyl*-(1→2)-*3,4*-O-*isopropylidene*-β-D-*galactopyranoside* (**11**).—To a soln of **10** (1.1 g, 1.03 mmol) in THF (10 mL) was added *n*Bu_4NF trihydrate (480 mg, 1.5 mmol). The mixture was stirred at rt for 5 h, then poured into cold water and extracted with EtOAc. The organic phase was washed with satd $NaHCO_3$, then dried over Na_2SO_4 and concd. Column chromatography (3:2 petroleum ether–EtOAc) of the residue gave **11** as a syrup (812 mg, 95%). $[\alpha]_D^{25}$ + 61° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3 H, CH_3), 1.07–1.25 (m, 20 H, 10 CH_2), 1.28, 1.54 (2 s, 6 H, 2 CH_3), 2.99 (bs, 1 H, O*H*), 3.30–3.34 (m, 1 H one proton of OCH_2R), 3.77–3.80 (m, 1 H, one proton of OCH_2R), 3.82–3.89 (m, 2 H, H-2, H-5), 3.90–4.00 (m, 2 H, H-6a, 6b), 4.25–4.30 (m, 3 H, $J_{1,2}$ 8.7 Hz, H-1, H-3, H-4), 4.71 (dd, 1 H, $J_{4',5'a}$ 5.5, $J_{5'a,5'b}$ 12.6 Hz, H-5′a), 4.77–4.82 (m, 2 H, $J_{4',5'b}$ 3.2 Hz, H-4′, H-5′b), 5.54 (d, 1 H, $J_{3',4'}$ 4.2 Hz, H-3′), 5.64 (s, 1 H, H-1′/H-2′), 5.72 (s, 1 H, H-2′/H-1′), 7.23–8.13 (m, 25 H, 5 Ph). Anal. Calcd for $C_{47}H_{60}O_{13}$: C, 67.79; H, 7.21. Found: C, 67.81; H, 7.27.

Dodecyl 2,3,4,6-tetra-O-*acetyl*-β-D-*galactopyranosyl*-(1→6)-*2,3,4-tri*-O-*acetyl*-β-D-*galactopyranosyl*-(1→6)-*3,4*-O-*isopropylidene*-β-D-*galactopyranoside* (**12**).—To a soln of **4c** (120 mg, 0.15 mmol) and **7** (60 mg, 0.15 mmol) in anhyd CH_2Cl_2 (15 mL) was added activated 4 Å molecular sieves. The mixture was cooled to −42 °C, then Me_3SiOTf (5.7 μL, 0.029 mmol) was added under N_2 protection. The mixture was stirred at rt for 1 h, neutralized with Et_3N (0.2 mL), and concd. Column chromatography (1:1 petroleum ether–EtOAc) of the residue gave **12** as a syrup (91 mg, 59%); $[\alpha]_D^{25}$ + 18° (*c* 0.2, $CHCl_3$); **12** was converted into its acetylated derivative, dodecyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2-*O*-acetyl-3,4-*O*-isopropylidene-β-D-galactopyranoside, showing the following physical data: $[\alpha]_D^{25}$ + 7° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, CH_3), 1.25 (bs, 18 H, 9 CH_2), 1.31 (s, 3 H, CH_3), 1.54 (s, 3 H, CH_3), 1.55–1.61 (m, 2 H, CH_2), 1.97, 1.98, 2.03, 2.06, 2.07, 2.09, 2.15, 2.16 (8 s, 24 H, 8 CH_3CO), 3.39–3.42 (m, 1 H, one proton of OCH_2), 3.78–3.94 (m, 7 H, H-6a, H-6b, one proton of OCH_2, H-6′a, H-6′b, H-5, H-5′), 4.07–4.10 (m, 5 H, H-5″, H-4, H-3, H-6″a, H-6″b), 8.28 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1), 4.55, 4.61 (2 d, 2 H, *J* 8.0 Hz, H-1′/H-1″), 4.91–5.00 (m, 3 H, H-2, H-3′, H-3″), 5.52–5.23 (m, 2 H, *J* 8.0, 10.8 Hz, H-2′/H-2″), 5.38 (d, 2 H, H-4′, H-4″). Anal. Calcd for $C_{49}H_{76}O_{24}$: C, 56.11; H, 7.25. Found: C, 56.02; H, 7.31.

Dodecyl 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→2)]-*3,4*-O-*isopropylidene*-β-D-*galactopyranoside* (**13**).—Method A. To a soln of **4c** (190 mg, 0.24 mmol) and **11** (202 mg, 0.24 mmol) in anhyd CH_2Cl_2 (15 mL) was added activated 4 Å molecular sieves. The mixture was cooled to −42 °C, then Me_3SiOTf (10 μL, 0.055 mmol) was added under N_2 protection. The mixture was stirred at rt for 1 h, neutralized with Et_3N (0.2 mL), then concd. Column chromatography (1:1 petroleum ether–EtOAc) of the residue gave **13** as a syrup (321 mg, 91%). Method B. The same procedure was used in the coupling reaction of **9b** (34 mg, 0.055 mmol) with **12** (50 mg, 0.05 mmol) to give **13** (53 mg, 74%); $[\alpha]_D^{25}$ − 16° (*c* 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3 H, CH_3), 1.07–1.33 (m, 18 H, 9 CH_2), 1.23, 1.53 (2 s, 6 H, 2 CH_3C), 1.97 (s, 6 H, 2 CH_3CO), 2.01, 2.05, 2.07, 2.14, 2.15 (5 s, 15 H, 5 CH_3CO), 3.33–3.38 (m, 1 H, one proton of OCH_2R), 3.80–3.93 (m, 8 H, H-2,4,5,6a,6b,6′a,6′b and one proton of OCH_2R), 4.04–4.17 (m, 4 H, H-3, H-5′, H-5″, H-6″a), 4.26–4.29 (m, 2 H, $J_{1,2}$ 8.8 Hz, H-1

and H-6″b), 4.54 (d, 1 H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.62 (d, 1 H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.71 (dd, 1 H, $J_{4,5a}$ 5.5, $J_{5a,5b}$ 11.2 Hz, H^{Ara}-5a), 4.78–4.80 (m, 2 H, H^{ara}-4, H^{ara}-5b), 4.97 (dd, 2 H, $J_{2',3'} = J_{2'',3''} = 10.5$, $J_{3',4'} = J_{3'',4''} = 3.3$ Hz, H-3′, H-3″), 5.18 (dd, 1 H, H-2′), 5.18 (dd, 1 H, H-2″), 5.37 (bs, 2 H, H-4′ and H-4″), 5.53 (d, 1 H, $J_{3,4}$ 4.6 Hz, H^{ara}-3), 5.61, 5.67 (2 s, 2 H, H^{ara}-1 and H^{ara}-2), 7.254–8.108 (m, 15 H, 3 Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1–31.9 (C^{Alkyl}), 61.2 (C^{Ara}-5), 63.8 (C-6″), 66.1 (C-6′), 66.9 (C-6), 67.4 (C-5″), 68.4 (C-4′), 68.9 (C-4″), 69.1 (CH_2O), 69.9 (C-5′), 70.8 (C-5), 70.9 (2 C, C-2″, C-3″), 72.0 (C-4), 74.9 (C-3), 78.1 (C-2), 79.4 (C-4), 81.5 (C-3), 81.7 (C-2), 100.6 (C-1″), 101.3 (C-1′), 101.4 (C-1), 104.2 (C^{Ara}-1), 110.6 (acetal C), 128.3–133.5 (C^{Ar}), 165.4, 165.8, 166.2 (3 Ph*C*O), 169.3, 169.4, 170.0, 170.04, 170.1, 170.2, 170.4 (7 CH_3CO). Anal. Calcd for $C_{73}H_{94}O_{30}$: C, 60.41; H, 6.48. Found: C, 60.51; H, 6.44.

Dodecyl 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→2)]-*3,4-di*-O-*acetyl*-β-D-*galactopyranoside* (**14**).—Compound **13** (950 mg, 0.66 mmol) was dissolved in aq 90% TFA (8 mL), and the mixture was stirred at rt. The reaction was monitored by TLC (3:2 petroleum ether–EtOAc) until all of the starting material was consumed (about 40 min). The mixture was diluted with toluene and then concd to dryness. After acetylation of the residue with Ac_2O (1 mL) in pyridine (2 mL), the soln was co-evaporated with toluene. Purification of the product on column chromatography (2:3 petroleum ether–EtOAc) gave pure **14** as a syrup (861 mg, 88%); $[\alpha]_D^{25} - 23°$ (*c* 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.86 (t, 3 H, CH_3), 1.07–1.54 (m, 20 H, 10 CH_2), 1.96, 1.98, 1.99, 2.03, 2.04, 2.05, 2.11, 2.14, 2.15 (9 s, 27 H, 9 CH_3CO), 3.32–3.35 (m, 1 H, one proton of OCH_2R), 3.66–3.92 (m, 7 H, H-5, H-5′, H-6a, H-6b, H-6′a, H-6′b, one proton of OCH_2R), 4.08 (dd, 1 H, $J_{1,2}$ 7.8, $J_{2,3}$ 10.5 Hz, H-2), 4.10–4.15 (m, 3 H, H-5″, H-6″a, H-6″b), 4.43 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.48, 4.50 (2 d, 2 H, $J_{1',2'}$ 7.9, $J_{1'',2''}$ 8.0 Hz, H-1′ and H-1″), 4.69–4.70 (dd, 1 H, *J* 4.6 and 11.4 Hz, H^{ara}-5a), 4.80–4.85 (m, 2 H, H^{ara}-4 and H^{ara}-5b), 4.95 (dd, 1 H, $J_{3',4'}$ 3.2 Hz, H-3′), 5.00 (dd, 1 H, $J_{3'',4''}$ 3.4 Hz, H-3″), 5.08 (dd, 1 H, $J_{3,4}$ 4.4 Hz, H-3), 5.12–5.16 (m, 2 H, H-2′ and H-2″), 5.36–5.39 (m, 4 H, H^{ara}-3, H-4′ and H-4″, H^{ara}-1/H^{ara}-2), 5.16 (s, 1 H, H^{ara}-2/H^{ara}-1), 5.59 (d, 1 H, H-4), 7.32–8.08 (m, 15 H, 3 Ph). Anal. Calcd for $C_{74}H_{94}O_{32}$: C, 59.44; H, 6.29. Found: C, 59.39; H, 6.27.

Dodecyl β-D-*galactopyranosyl*-(1→6)-β-D-*galactopyranosyl*-(1→6)-[α-L-*arabinofuranosyl*(1→2)]-β-D-*galactopyranoside* (**15**).—Compound **14** (810 mg, 0.54 mmol) was treated with NaOMe in MeOH (keep pH at 9) at rt for 24 h, then neutralized with Dowex-50W (H^+) resin and concd. One portion of the product (from about 230 mg of **14**) was dissolved in 1:1 MeOH–water (10 mL) and transferred into a dialysis tubing (with molecular-weight cut-off 500). The soln was dialyzed for 48 h and then concd under reduced pressure to give a crude product **15** (44 mg, 35%). Another portion of the fully deacylated product (from about 500 mg of **14**) was purified directly on Sephadex LH-20 column chromatography using MeOH as the eluent to afford **15** (214 mg, 80%); $[\alpha]_D^{25} - 6°$ (*c* 1.5, 5:1 MeOH–H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 0.8–1.4 (m, 23 H, alkyl), 3.30–3.40 (m, 2 H), 3.60–4.12 (m, 34 H), 4.20 (bs, 1 H, H^{Ara}-2), 4.31 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.49 (d, 1 H, *J* 7.4 Hz, H-1′/H-1″), 4.54 (d, 1 H, *J* 7.6 Hz, H-1″/H-1′), 5.25 (bs, 1 H, H^{Ara}-1). ^{13}C NMR (MeOD:D_2O, 4:1, 100 MHz): δ 13.5–31.6 (11 C, $OCH_2(CH_2)_{10}CH_3$), 62.2 (C-6″), 63.2 (C^{Ara}-5), 69.9, 69.8 (C-6, C-6′), 70.9, 71.6, 72.1, 74.1, 74.3, 74.4, 74.8, 74.9, 75.0, 76.4, 76.7, 77.0, 78.0, 80.0, 83.3, 86.8 (C-2,3,4,5, C-2′,3′,4′,5′, C-2″, 3″, 4″,5″, C^{Ara}-2, C^{Ara}-3, C^{Ara}-4, CH_2O), 104.2, 105.2, 105.8 (C-1, C-1′, C-1″), 110.6 (C^{Ara}-1). ESMS Calcd for $C_{35}H_{64}O_{20}$ (804). Found ESIMS (+): 827.6 $[M + Na]^+$; ESIMS (−): 803.7 $[M]^-$.

Acknowledgements

This work was supported by The Chinese Academy of Sciences (Project KJ952J$_1$510) and The National Natural Science Foundation of China (Projects 29672049 and 29802009). Y.D. also acknowledges the support from SRF for ROCS, SEM.

References

[1] W.T. Willats, J.P. Knox, *Plant J.*, 9 (1996) 919–921.
[2] (a) R.I. Pennell, J.P. Knox, G.N. Scofield, R.R. Selvendran, K. Robert, *J. Cell Biol.*, 108 (1989) 1967–1975. (b) J.P. Knox, P.J. Linstead, J. Peart, C. Cooper, K. Roberts, *Plant J.*, 1 (1991) 317–323.
[3] (a) H. Yamada, H. Kiyohara, J.-C. Cyong, Y. Otsuka, *Carbohydr. Res.*, 159 (1987) 275–291. (b) H. Yamada, *Carbohydr. Polymers*, 25 (1994) 269–274.
[4] (a) J.-F. Valdor, W. Mackie, *J. Carbohydr. Chem.*, 16 (1997) 429–440. (b) C.M. Timmers, S.C.M. Wigchert, M.A. Leeuwenburgh, G.A. van der Marel, J.H. Boom, *Eur. J. Org. Chem.*, (1998) 91.
[5] W. Steffan, P. Kovac, P. Albersheim, A.G. Darvill, M.G. Hahn, *Carbohydr. Res.*, 275 (1995) 295–307.
[6] J.P. Knox, *Protoplasma*, 167 (1992) 1–9.
[7] W. Koenigs, E. Knorr, *Berichtung*, 34 (1901) 957–962.
[8] C. Salat, P.A. Agneseti, M.B. Caniato, Spanish Patent No. 430 636 (1976); *Chem. Abstr.*, 87 (1987) 23683k.
[9] E.A. Talley, *Methods Carbohydr. Chem.*, 2 (1963) 337–340.
[10] H.G. Fletcher Jr., *Methods Carbohydr. Chem.*, 2 (1963) 228–230.
[11] G. Yang, F. Kong, unpublished results.
[12] R.R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 50 (1994) 21–124.
[13] W. Wang, F. Kong, *J. Org. Chem.*, 63 (1998) 5744–5745.
[14] P.J. Garegg, R. Johansson, B. Samuelsson, *Acta. Chem. Scand., Ser. B*, 36 (1982) 249–250.