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First synthesis of 5,6-branched galacto-hexasaccharide, the dimer of the trisaccharide repeating unit of the cell-wall galactans of *Bifidobacterium catenulatum* YIT 4016

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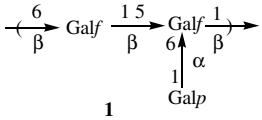
Abstract—α-D-Galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→5)]-β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-[α-D-galactopyranosyl-(1→6)]-β-D-galactofuranose, the dimer of the trisaccharide repeating unit of the cell-wall galactans of *Bifidobacterium catenulatum* YIT 4016, has been synthesized as its dodecyl glycoside **2** by coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranosyl trichloroacetimidate **14** with dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranoside **16**. The trisaccharide trichloroacetimidate donor **14** and trisaccharide acceptor **16** were regiospecifically prepared by employing 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose **4** as the glycosyl acceptor, and isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **5** and 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate **9** as glycosyl donors.

1. Introduction

Recent demonstrations that oligosaccharides play important roles in diverse biological events have resulted in renewed interest in the synthesis of oligosaccharides.[1,2] However, compared with other biopolymers such as peptides and nucleic acids, the role of oligosaccharide structure in function has been minimally studied owing to the wide structural and stereochemical diversification. Although many advances have been made over recent decades,[3] the clear facts remain that we have singularly failed to develop a general solution and glycosylation chemistry is still not predictable or generally accessible. To facilitate the synthesis of target oligosaccharides, regioselective glycosylation of glycosyl donors with unprotected or partially protected sugar acceptors has been extensively studied. Employing this strategy, in the last few years, we have prepared a lot of oligosaccharides with various structures present in natural sources such as 3,6-branched gluco-oligosaccharides,[4] 2,6-branched manno-oligosaccharides,[5] 2,6-branched,[6] 3,6-branched and 5,6-branched galacto-oligosaccharides.[7,8]

Bifidobacterium, a bacteria living in the mammalian intestines is thought to be helpful in the protection of hosts from bacterial infections, suppression of enteric putridity and the supply of several kinds of vitamin, etc.[9] As the major components of the cell wall, the galactans play important roles in biological activities. In 1996, the structure of the galactan **1** from the cell wall of *Bifidobacterium catenulatum* YIT 4016 was elucidated by Nagaoka et al. (Fig. 1).[10] This galactan is composed



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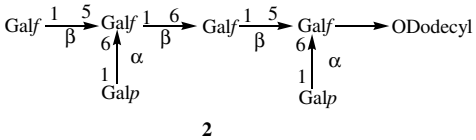

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Figure 1. Cell-wall galactan of *Bifidobacterium catenulatum* YIT 4016 **1** and the synthesized hexasaccharide **2**.

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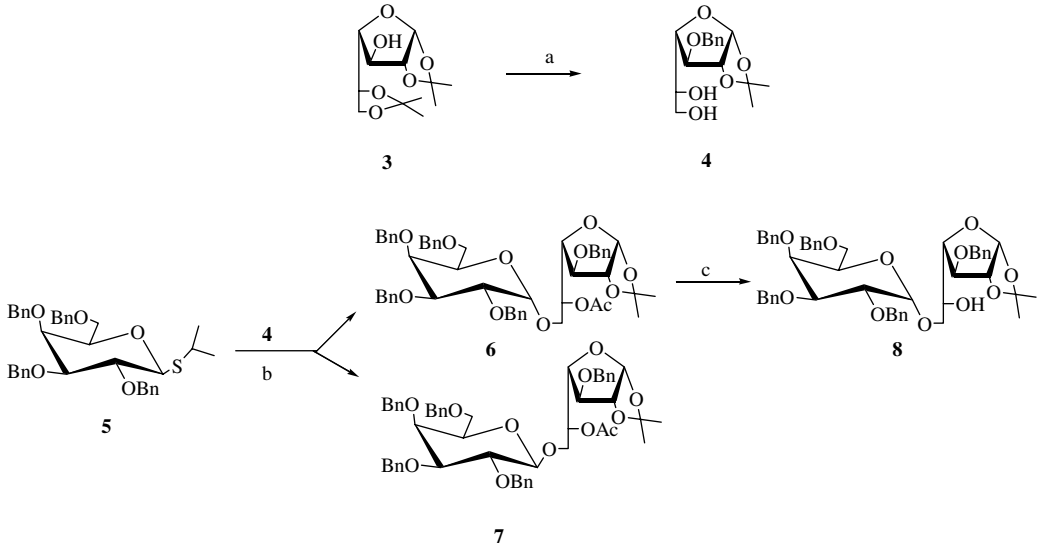
of β(1→5) linked galactofuranose residues, while the branches consist of galactopyranosyl residues. From a synthetic standpoint, this structure is extremely complicated because both the 5- and 6-positions of the reducing galactofuranose component are linked with galactofuranosyl and galactopyranosyl residues, and the glycosidic linkage of the galactopyranose moiety is α.

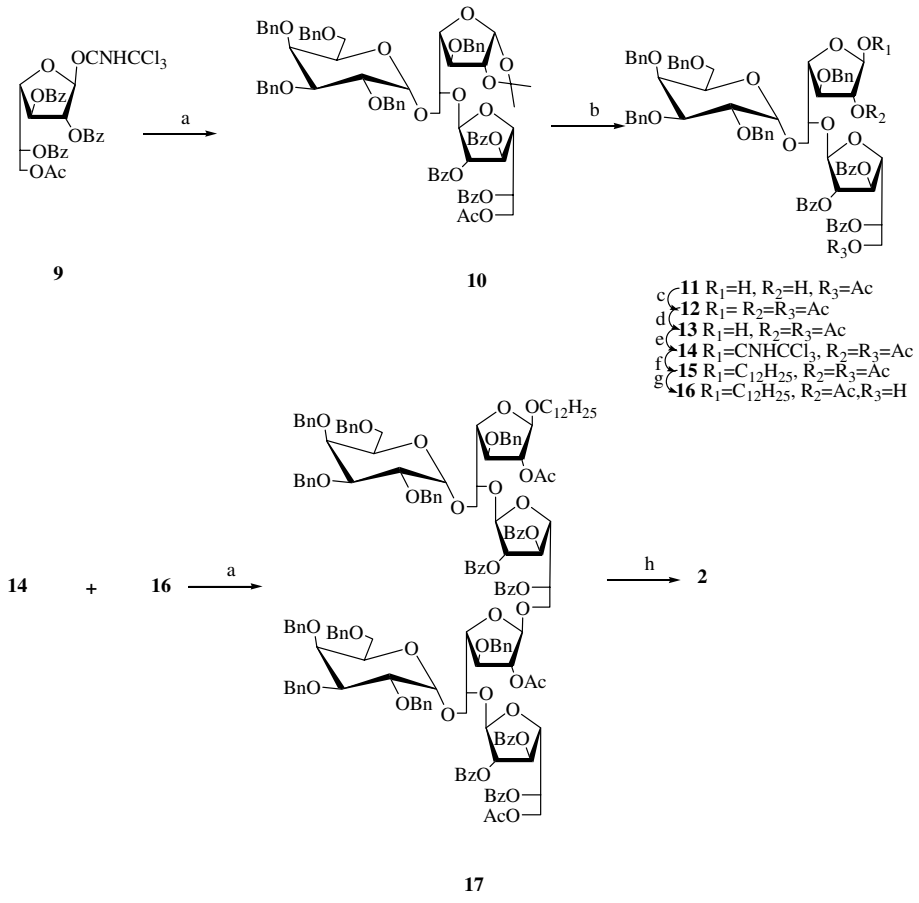
In addition to the extremely complex structure, there are other interests promoting us to synthesize the oligomer of this trisaccharide repeating unit. First, the backbone of the β(1→5) linked galactofuranose chain is of immunological importance. For example, studies on *Aspergillus* cell wall antigens showed that the β(1→5) linked galactofuranose chains were regarded to be the immunodominant etitopes.[11] Second, the fact that galactofuranose residues have not been found in mammalian glycoconjugates implied that these oligosaccharides could be potential diagnostic agent and therapeutic drug for intestinal disease that should have limited side effect in mammalian cells.[12] Third, the synthesis of target compound is useful for further elucidating the molecular structure responsible for the biological activities. These, together with the fact that synthesis of this kind of oligosaccharide has not been reported so far, promote us to develop a method for the synthesis of the dimer hexasaccharide of the trisaccharide repeating unit of the cell-wall galactans of *Bifidobacterium catenulatum* YIT 4016.

2. Results and discussion

Preparation of oligosaccharides is one of the most difficult and complex tasks in organic chemistry. Most glycosylation methods are extremely sensitive to structural variations in the glycosyl donor–acceptor pairs.[13] Reaction conditions that provide excellent yields with one donor–acceptor pair may give virtually no product for another donor–acceptor pair. Furthermore, the stereochemical outcome is often difficult to predict. In our synthesis, 1,2:5,6-di-*O*-isopropylidene-α-D-galactofuranose **3** was a key starting material, which was prepared by our improved method.[14] Benzylation of **3** in DMF with $PhCH_2Br$ at rt, followed by selective 5,6-*O*-deacetonation with 90% AcOH at 40 °C afforded 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose **4** (Scheme 1). For stereoselectively forming α-galactopyranosides in glycosylation reactions, a general method was using the glycosyl donor possessing a benzyl group at the C-2 position. However, when we used the same rule to synthesize our desired α-(1→6)-linked galactodisaccharide we found that there was nearly no stereoselectivity. Coupling of **4** with isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **5**[15] gave a mixture consisting of α-(1→6)-linked and β-(1→6)-linked disaccharides in a ratio of 1:1.25. What made the thing more difficult was that the mixture of the two disaccharides could not be separated. Fortunately, we found that after acetylation of the α- and β-linked disaccharides, the desired product, 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose **6** could be isolated in a poor yield (27%). Through comparison with the spectrum of its isomer, 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→6)-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose **7**, the structure of compound **6** was unambiguously identified by ^1H NMR data. The characteristic resonances due to the anomeric protons H-1α, H-1β were located as a doublet at 4.94 ppm with $J_{1,2} = 3.7$ Hz and as a doublet at 4.40 ppm with $J_{1,2} = 7.6$ Hz, respectively. Deacylation of **6** in ammonia-saturated methanol furnished **8**. Coupling of 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate **9**[16–18] and **8** with TMSOTf as catalyst in CH_2Cl_2 at rt gave 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-3-*O*-benzyl-1,2-*O*-isopropylid-



Scheme 1. Reagents and conditions: (a) i. $PhCH_2Br$, NaH, DMF, rt, overnight; ii. 90% HOAc, 40 °C, 24 h, 92% (over two steps); (b) i. **4**, CH_2Cl_2, TMSOTf (cat.), NIS, rt, 1 h; ii. Ac_2O, pyridine, rt, 2 h, 27% for **6** and 34% for **7** (over two steps); (c) CH_2Cl_2–CH_2OH saturated with ammonia, rt, 12 h, 92%.



Scheme 2. Reagents and conditions: (a) CH_2Cl_2, TMSOTf (cat.), rt, 1 h, 95% for **10**, 91% for **17**; (b) 10:1 $CHCl_3$–CF_3COOH, rt, 2 h; (c) Ac_2O, pyridine, rt, 2 h; (d) benzylamine, THF, under darkness, rt, 24 h; (e) CCl_3CN, CH_2Cl_2, K_2CO_3, rt, 12 h, 75% (over four steps); (f) $C_{12}H_{25}OH$, TMSOTf (cat.), CH_2Cl_2, rt, 1 h, 97%; (g) 0.5% HCl in MeOH, rt, 10 h, 56%; (h) i. 1:4 MeOH/EtOAc, Pd/C (5%), rt, 24 h; ii. saturated with dry NH_3, rt, 24 h, 80% (over two steps).

ene-α-D-galactofuranose **10** in 95% yield. The structure of **10** was confirmed by ^{1}H NMR and ^{13}C NMR data. ^{1}H NMR revealed three anomeric proton signals at 5.82 ppm as a doublet with $J_{1,2}$ = 4.1 Hz, 4.84 ppm as a doublet with $J_{1,2}$ = 3.6 Hz and 5.66 ppm as a singlet, meanwhile the ^{13}C NMR spectrum revealed anomeric carbons at 105.16, 104.63 and 97.88 ppm (Scheme 2).

De-isopropylidenation of **10** in 10:1 $CHCl_3$–CF_3COOH (v/v) at rt offered **11**. Acetylation of **11** with acetic anhydride in pyridine gave **12**. Selective 1-*O*-deacetylation of **12** with $PhCH_2NH_2$ in THF gave **13**. After treating **13** with trichloroacetonitrile in the presence of K_2CO_3, the desired trisaccharide glycosyl donor **14** was obtained. Coupling of **14** with $C_{12}H_{25}OH$ gave **15**. There was no difficulty for selective 6-*O*-deacetylation of **15** in MeOH solution containing 0.5% HCl to give the glycosyl acceptor **16** in an acceptable yield (56%). Condensation of **14** and **16** offered dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranoside **17**. The ^{1}H NMR spectrum of **17** contained structurally characteristic information: three acetyl signals (δ 1.89, 1.80 and 1.70 ppm). In addition, the chemical shifts of anomeric carbons of **17** revealed by ^{13}C NMR spectrum were at 106.34, 106.20, 106.07, 105.75, 97.94 and 97.81 ppm. Finally, deprotection of **17** yielded the target compound **2**. The ^{13}C NMR spectrum of **2** gave six signals for C-1 (108.03, 107.51, 107.40, 107.11, 98.50 and 98.50 ppm) confirming the structure of **2**.

3. Conclusion

In summary, synthesis of the dimer of the trisaccharide repeating unit of the cell-wall galactans of *Bifidobacterium catenulatum* YIT 4016 has been achieved for the first time by regioselective glycosylation using partially protected sugars as the acceptor. This should promote the studies on fundamental biochemical properties and biological functions about this oligosaccharide.

4. Experimental section

4.1. General methods

Optical rotations were determined at 25 °C with a digital polarimeter. The NMR spectra were recorded in $CDCl_3$ with TMS internal standard or D_2O with ethanol as

standard on ARX 400 MHz. Mass spectra were recorded on an autospec mass spectrometer using ESI technique to introduce the sample.

Elemental analyses were done on elemental analyzer model 1108 EA. 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. Dry solvents were distilled over CaH_2 and stored over molecular sieves.

4.2. 3-*O*-Benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose 4

To a solution of **3** (3.0 g, 12 mmol) in dry DMF (80 mL) was added $PhCH_2Br$ (1.5 mL, 13 mmol) and NaH (0.50 g) at 0 °C. After stirring the mixture overnight at rt, TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. Water (10 mL) was added to the reaction mixture, and then the mixture was diluted with EtOAc and washed with water. The organic phase was concentrated, and the resulting residue was directly dissolved in 90% acetic acid solution (80 mL). The mixture was kept at 40 °C for 24 h and then concentrated to a residue under reduced pressure. The residue was purified by column chromatography (3:1 petroleum ether–EtOAc) to afford **4** (3.3 g, 92% for two steps): $[\alpha]_D = -11.2$ (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.36–7.25 (m, 5H, Ph*H*), 5.91 (d, 1H, $J = 4.1$ Hz, H-1), 4.68 (d, 1H, $J = 3.5$ Hz, H-3), 4.67–4.54 (m, 2H, PhCH_2), 4.14–4.11 (m, 1H, H-4), 4.00 (d, 1H, $J = 2.8$ Hz, H-2), 3.80–3.77 (m, 1H, H-5), 3.70–3.67 (dd, 1H, $J = 3.7$ Hz, 11.7 Hz, H-6a), 3.60–3.56 (dd, 1H, $J = 4.8$ Hz, 11.7 Hz, H-6b), 2.55 (s, 1H, O*H*), 2.13 (s, 1H, O*H*), 1.52 (s, 3H, (CH_3)C), 1.34 (s, 3H, (CH_3)C). Anal. Calcd for $C_{16}H_{22}O_6$: C, 61.92; H, 7.15. Found: C, 61.52; H, 7.34.

4.3. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose 6

A solution of isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **5** (6.3 g, 10.5 mmol) and 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose **4** (3.2 g, 10.3 mmol) in anhydrous CH_2Cl_2 (40 mL) was stirred with activated 4 Å molecular sieves (2 g) and NIS (2.3 g, 9.4 mmol) at rt for 20 min, and then TMSOTf (15 μL) was added. The mixture was stirred for 1 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. The residue was acetylated with acetic anhydride (3 mL) in pyridine (30 mL) for 2 h at rt and the mixture was concentrated to dryness. Purification of the residue by silica gel column chromatography (6:1 petroleum ether–EtOAc) gave solid **6** (2.5 g, 27%): $[\alpha]_D = +12.8$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.35–7.25 (m, 25H, 5Ph*H*), 5.72 (d, 1H, $J = 4.1$ Hz, H-1′), 5.32–5.28 (m, 1H, H-5′), 4.94 (d, 1H, $J = 3.7$ Hz, H-1), 4.93–4.25 (m, 12H, H-3′, H-4′, 5PhCH_2), 4.05–4.01 (dd, 1H, $J = 3.7$ Hz, 9.8 Hz, H-6a′), 3.93–3.86 (m, 4H, H-6b′, H-2′, H-2, H-4), 3.83–3.79 (dd, 1H, $J = 6.0$ Hz, 10.8 Hz, H-6a), 3.69–3.64 (dd, 1H, $J = 5.6$ Hz, 11.0 Hz, H-6b), 3.50–3.48 (m, 2H, H-5, H-3), 1.98 (s, CH_3CO), 1.53 (s, 3H, (CH_3)C), 1.35 (s, 3H, (CH_3)C). Anal. Calcd for $C_{52}H_{58}O_{12}$: C, 71.38; H, 6.68. Found: C, 71.83; H, 6.77.

4.4. 2,3,4,6-Tetra-*O*-benzyl-β-D-galactopyranosyl-(1→6)-5-*O*-acetyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose 7

$[\alpha]_D = +10.8$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.30 (m, 25H, 5Ph*H*), 5.82 (d, 1H, $J = 4.0$ Hz, H-1′), 5.44–5.43 (m, 1H, H-5′), 4.98–4.41 (m, 11H, 5PhCH_2, H-4′), 4.40 (d, 1H, $J = 7.6$ Hz, H-1), 4.15–4.11 (m, 2H, H-4, H-2′), 3.97–3.94 (m, 2H, H-2, H-3′), 3.87–3.84 (dd, 1H, $J = 6.9$ Hz, 10.6 Hz, H-6a), 3.75–3.72 (dd, 1H, $J = 6.9$ Hz, 10.6 Hz, H-6b), 3.63–3.53 (m, 4H, H-3, H-5, 2H-6′), 1.97 (s, 3H, CH_3CO), 1.60 (s, 3H, CH_3(CH)), 1.41 (s, 3H, CH_3(CH)). Anal. Calcd for $C_{52}H_{58}O_{12}$: C, 71.38; H, 6.68. Found: C, 71.89, H, 6.78.

4.5. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-galactofuranose 8

Compound **6** (2.4 g, 2.7 mmol) was dissolved in an ammonia-saturated solution of 1:10 CH_2Cl_2–CH_3OH (55 mL) saturated with NH_3 at rt. After 12 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and the residue was passed through a silica gel column with 3:1 petroleum ether–EtOAc as the eluent to give **8** (2.1 g, 92%): $[\alpha]_D = +14.7$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.35–7.23 (m, 25H, 5Ph*H*), 5.85 (d, 1H, $J = 4.1$ Hz, H-1′), 4.93–4.36 (m, 13H, 5PhCH_2, H-1, H-3′, H-4′), 4.11–3.91 (m, 5H, H-6a′, H-6b, 2H-2, H-4), 3.75–3.72 (dd, 1H, $J = 6.0$ Hz, 10.8 Hz, H-6a), 3.60–3.56 (dd, 1H, $J = 4.3$ Hz, 11.0 Hz, H-6b′), 3.52–3.44 (m, 2H, H-3, H-5), 2.96 (d, 1H, $J = 5.6$ Hz, H-5′), 1.53 (s, 3H, C(CH_3)), 1.34 (s, 3H, C(CH_3)). Anal. Calcd for $C_{50}H_{56}O_{11}$: C, 72.10; H, 6.78. Found: C, 71.86; H, 6.69.

4.6. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-3-*O*-benzyl-α-1,2-*O*-isopropylidene-D-galactofuranose 10

A solution of 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl trichloroacetimidate **9** (1.7 g, 2.5 mmol) and **8** (2.0 g, 2.4 mmol) in anhydrous CH_2Cl_2 (40 mL) was stirred with activated 4 Å molecular sieves at rt for 20 min, and then TMSOTf (15 μL) was added. The mixture was stirred for 1 h, at the end of which time TLC (2.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N, filtered and then the filtrate was con-

centrated. The resultant residue was subjected to the column chromatography with 2.5:1 petroleum ether–EtOAc as the eluent to give **10** (3.1 g, 95%): $[\alpha]_D = +2.5$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–7.11 (m, 40H, 8Ph*H*), 6.00–5.96 (m, 1H, H-5″), 5.82 (d, 1H, *J* = 4.1 Hz, H-1′), 5.66 (s, 1H, H″-1), 5.52 (d, 1H, *J* = 9.2 Hz, H-3″), 5.45 (s, 1H, H-2″), 4.92–4.84 (dd, 1H, *J* = 2.7 Hz, 5.3 Hz, H-4″), 4.88 (d, 1H, *J* = 8.4 Hz, H-3′), 4.85 (d, 1H, *J* = 3.6 Hz, H-1), 4.74–4.36 (m, 13H, 5PhCH_2, H-5′, H-2, H-4), 4.14–3.81 (m, 7H, 4H-6, H-4′, H-3, H-2′), 3.60–3.47 (m, 3H, H-5, 2H-6), 1.97 (s, 3H, CH_3CO), 1.61 (s, 3H, C(CH_3)), 1.34 (s, 3H, C(CH_3)). Anal. Calcd for $C_{79}H_{80}O_{20}$: C, 70.31; H, 5.98. Found: C, 70.62; H, 6.15.

4.7. 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*- benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranosyl trichloroacetimidate 14

Compound **10** (3.1 g, 2.3 mmol) was treated with 10:1 $CHCl_3$–CF_3COOH (50 mL) at rt for 2 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was diluted with toluene (100 mL), and the mixture was concentrated under vacuum, and then the residue was acetylated with acetic anhydride (5 mL) in pyridine (50 mL) for 2 h at rt. The resultant trisaccharide and benzylamine (5 mL) in anhydrous THF (100 mL) was kept under darkness at rt for 24 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 (100 mL), and was washed with 1 N HCl and saturated aq $NaHCO_3$. The combined organic phrase was dried (Na_2SO_4) and concentrated to dryness. The resultant residue without purification was dissolved in CH_2Cl_2 (50 mL), and then CCl_3CN (0.55 mL, 5.5 mmol) and K_2CO_3 (2.5 g) were added. The mixture was stirred for 12 h at rt, and the solid material was filtrated off. Concentration of the filtrate, followed by purification on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent, gave the trisaccharide donor **14** (2.6 g, 75%): $[\alpha]_D = -1.5$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H, O(CN*H*)CCl_3), 7.92–7.14 (m, 40H, 8Ph*H*), 6.28 (s, 1H, H-1′), 5.91 (m, 1H, H-5″), 5.66 (s, 1H, H-1″), 5.65 (d, 1H, *J* = 8.4 Hz, H-3″), 5.50 (s, 1H, H-2″), 5.47 (d, 1H, *J* = 8.4 Hz, H-3′), 5.38 (s, 1H, H-2′), 4.87–4.25 (m, 15H), 4.05–3.87 (m, 6H, 4H-6, H-4, H-3), 3.58–3.33 (m, 3H, H-5, 2H-6), 2.04 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO). Anal. Calcd for $C_{80}H_{78}Cl_3NO_{21}$: C, 64.24; H, 5.26. Found: C, 64.67; H, 5.41.

4.8. Dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)]-6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranoside 15

A solution of **14** (2.0 g, 1.3 mmol) and $C_{12}H_{25}OH$ (0.37 g, 2.0 mmol) in anhydrous CH_2Cl_2 (40 mL) was stirred with activated 4 Å molecular sieves (2 g) at rt for 20 min, and then TMSOTf (15 μL) was added. The mixture was stirred for 1 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. Then the mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (3:1 petroleum ether–EtOAc) gave **15** (2.0 g, 97%) as a syrup: $[\alpha]_D = -8.6$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.05–7.17 (m, 40H, 8Ph*H*), 5.92–5.88 (m, 1H, H-5″), 5.70 (s, 1H, H-1′), 5.54 (s, 1H, H-1″), 5.52 (d, 1H, *J* = 9.2 Hz, H-3″), 5.10 (s, 1H, H-2), 4.95 (s, 1H, H-2″), 4.88–4.21 (m, 16H), 4.11–4.00 (m, 3H), 3.94–3.86 (m, 3H), 3.61–3.25 (m, 5H, 3H-6, C$H_2C_{12}H_{23}$), 1.98 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.59–0.86 (m, 23H, $CH_2C_{11}H_{23}$). Anal. Calcd for $C_{90}H_{102}O_{21}$: C, 71.13; H, 6.76. Found: C, 70.92; H, 6.98.

4.9. Dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)]-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranoside 16

Acetyl chloride (0.25 mL) was added to a solution of compound **15** (1.5 g, 0.99 mmol) in CH_3OH (50 mL) and CH_2Cl_2 (2 mL), and the reaction was carried out at rt for 10 h. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. After neutralization and concentration, the residue was subjected to column chromatography on silica gel using petroleum ether–EtOAc (2:1) as the eluent to give **16** (0.82 g, 56%): $[\alpha]_D = -15.6$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–7.20 (m, 40H, 8Ph*H*), 5.72 (s, 1H, H-1′), 5.62–5.59 (m, 2H, H-5, H-1), 5.53 (d, 1H, *J* = 9.2 Hz, H-3″), 5.11 (s, 1H, H-2′), 4.98–3.26 (m, 28H), 2.00 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.59–0.84 (m, 23H, $CH_2C_{11}H_{23}$). Anal. Calcd for $C_{88}H_{100}O_{20}$: C, 71.52; H, 6.82. Found: C, 71.78; H, 6.76.

4.10. Dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)-[6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→5)-[2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-(1→6)]-2-*O*-acetyl-3-*O*-benzyl-β-D-galactofuranoside 17

A solution of **14** (0.55 g, 0.37 mmol) and **16** (0.50 g, 0.34 mmol) in anhydrous CH_2Cl_2 (50 mL) was stirred with activated 4 Å molecular sieves (2 g) at rt for 20 min, and then TMSOTf (12 μL) was added. The mixture was stirred for 1 h, at the end of which time TLC (1.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (1.5:1 petroleum ether–EtOAc) gave **17** (0.87 g, 91%): $[\alpha]_D = -13.4$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.03–7.01 (m, 80H, 16Ph*H*), 5.85 (m, 1H, H-5), 5.74 (m, 1H, H-5), 5.70 (s, 1H, H-1), 5.69 (s, 1H, H-1), 5.67–5.57 (m, 2H, H-1, H-2), 5.50–5.48 (m, 2H, H-1, H-2), 5.12–3.20 (m, 56H), 1.89 (s, 3H, CH_3CO), 1.80 (s, 3H, CH_3CO), 1.70 (s, 3H, CH_3CO), 1.26–0.87 (m, 23H, $CH_2C_{11}H_{23}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.52, 169.96, 169.40 (3CH_3CO), 165.68, 165.68, 165.59, 165.51, 165.17, 165.17 (6Ph*C*O), 106.34, 106.20, 106.07, 105.75 (4β-C-1), 97.94, 97.81 (2α-C-1).

Anal. Calcd for $C_{166}H_{176}O_{40}$: C, 70.92; H, 6.31. Found: C, 70.58; H, 6.47.

4.11. Dodecyl α-D-galactopyranosyl-(1→6)-[β-D-galactofuranosyl-(1→5)]-β-D-galactofuranosyl-(1→6)-β-D-galactofuranosyl-(1→5)-[α-D-galactopyranosyl-(1→6)]-β-D-galactofuranoside 2

To a solution of **17** (0.50 g, 0.18 mmol) in 1:4 MeOH/EtOAc (40 mL) was added Pd/C (5%, 40 mg). The reaction mixture was stirred for 24 h at rt in hydrogen atmosphere, at the end of which time TLC indicated that the debenzylation of **17** was complete. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to dryness. The residue was dissolved in a saturated solution of NH_3 in anhydrous CH_3OH (10 mL). After 24 h at rt, the reaction mixture was concentrated to about 5 mL, and then CH_2Cl_2 (40 mL) was added. The resultant precipitate was filtered and washed with CH_2Cl_2 (4×5 mL) to afford **2** (0.16 g, 80%): $[\alpha]_D = +2.4$ (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.26–4.74 (m, 6H, 6H-1), 1.28–1.12 (m, 23H, $CH_2C_{11}H_{23}$); ^{13}C NMR (100 MHz, D_2O): δ 108.03, 107.51, 107.40, 107.11 (4β-C-1), 98.50, 98.50 (2α-C-1). Anal. Calcd for $C_{48}H_{86}O_{31}$: C, 49.73; H, 7.48. Found: C, 49.97; H, 7.35. MALDI-TOF MS: Calcd for $C_{48}H_{86}O_{31}$, 1159.18 [M]. Found: 1182.30 $(M+Na)^+$.

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References

1. Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.
2. *Synthetic Oligosaccharides. Indispensable Probes for the Life Science*; Kovac, P., Ed.; ACS Symposium Series; American Chemical Society: Washington, DC, 1994; p 560.
3. (a) Plant, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523; (b) Sears, P.; Wong, C. H. *Science* **2001**, *291*, 2344.
4. (a) Ning, J.; Yi, Y.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 5545–5549; (b) Ning, J.; Zhang, W.; Yi, Y.; Yang, G.; Wu, Z.; Yi, Z.; Kong, F. *Bioorg. Med. Chem.* **2003**, *11*, 2193–2203.
5. (a) Xing, Y.; Ning, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1275–1283; (b) Ning, J.; Heng, L.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 673–675.
6. Ning, J.; Yi, Y.; Yao, Z. *Synlett* **2003**, *14*, 2208–2212.
7. Ning, J.; Wang, H.; Yi, Y. *Tetrahedron Lett.* **2002**, *43*, 7349–7352.
8. Wang, H.; Ning, J. *J. Org. Chem.* **2003**, *68*, 2521–2524.
9. Deguchi, Y.; Morishita, T.; Mutai, M. *Agric. Biol. Chem.* **1985**, *49*, 13–19.
10. Nagaoka, M.; Hashimoto, S.; Shibata, H.; Kimura, I.; Kimura, K.; Sawada, H.; Yokokura, T. *Carbohydr. Res.* **1996**, *281*, 285–291.
11. Leitao, E. A.; Bittencourt, V. C. B.; Haido, R. M. T.; Valente, A. P.; Peter-Katalinic, J.; Letzel, M.; Souza, L. M.; Barreto-Bergter, E. *Glycobiology* **2003**, *13*, 681–692.
12. Lederkremer, R. M.; Marino, C.; Varela, O. *Carbohydr. Res.* **1990**, *200*, 227–235.
13. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1500–1562.
14. Wang, H.; Zhang, G.; Ning, J. *Carbohydr. Res.* **2003**, *338*, 1033–1037.
15. Du, Y.; Zhang, M.; Yang, F.; Gu, G. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3122–3127.
16. Tsvetkov, Y. E.; Nikolaev, A. V. *J. Chem. Soc., Perkin Trans. 1* **2000**, 889–891.
17. Sarkar, S. K.; Chouhury, A. K.; Mukhopadhyay, B.; Boy, N. *J. Carbohydr. Chem.* **1999**, *18*, 1121–1130.
18. Sarkar, S. K.; Roy, N. *J. Carbohydr. Chem.* **2003**, *22*, 285–296.