Carbohydrate Research 345 (2010) 2272–2276

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Synthesis of the pentasaccharide repeating unit of *Escherichia coli* O128 antigen

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ARTICLE INFO

Article history:
Received 15 June 2010
Received in revised form 9 July 2010
Accepted 14 July 2010
Available online 21 July 2010

Keywords:
Glycosylation
Escherichia coli O128 antigen
Oligosaccharides

ABSTRACT

A pentasaccharide, 4-methoxyphenyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→6)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranoside (**1**), representing the repeating unit of *Escherichia coli* O128 antigen, was successfully prepared in 23% overall yield via a convergent '2+3' glycosylation strategy.

The O-antigens (O-specific polysaccharides) are the exposed part of the lipopolysaccharides (LPS), the main outer-membrane component of Gram-negative bacteria. Structural variations of O-antigens contribute to the wide variety of antigenic types of different bacterial species.[1] O-antigens are furthermore recognized as important virulence factors and have the potential to influence host–pathogen interactions in many ways.[2] During the investigation on the molecular basis related to infantile diarrhea, *E. coli* O128 antigen has been isolated and characterized from enteropathogenic *E. coli* strains, one of the major species associated with diarrhea patients worldwide.[3] The primary structure of the O-antigen repeating unit of *E. coli* O128 has been established[4] as →3)-β-D-GalNAc-(1→4)-α-D-Gal-(1→3)-β-D-GalNAc-(1→6)-[α-L-Fuc-(1→2)]-β-D-Gal-(1→, a pentasaccharide (Fig. 1) in which [α-L-Fuc-(1→2)]-β-D-Gal was proposed to be the immunodominant part. Due to frequently occurring antibiotic-resistant pathogens, there are stringent requirements in developing potent vaccines against infectious diseases. Vaccines consisting of carbohydrates coupled to a carrier protein have been found to be effective for the prevention of bacterial invasive diseases.[5,6] Thus, providing structurally defined glycoconjugates via synthetic carbohydrate antigens would be one of the attractive strategies in healing infectious diseases.[7,8] As a collaborative project, we envisioned that the efficient synthesis of the pentasaccharide repeating unit of the O-128 antigen would pave way for the development of a well-structured glycoconjugate vaccine against infantile diarrhea *E. coli* O128. We herein report the synthesis of the core pentasaccharide using a convergent '2+3' glycosylation strategy.

In our previous work, we found that using a β-(1→4)-linked disaccharide donor might generate an α-glycosidic product under proper glycosylation conditions.[9] Accordingly, *E. coli* O128 repeating pentasaccharide could be disconnected into a disaccharide donor **2**, in which the 2-OH was blocked with a non-neighboring participation group such as benzyl, and a trisaccharide acceptor **3**. Both **2** and **3** could be assembled from known or commercially available monosaccharide building blocks **4–8** (Scheme 1).

In the preparation of disaccharide donor **2**, we first tried the coupling reaction between compounds **4**[10] and **5**[11] with the traditional method.[12] Unfortunately, we observed a significant amount of byproduct formed via thio-group transfer[13–15] from **5** to **4**. We thus turned our attention to the 'inverse procedure'[16] under low temperature (Scheme 2), in which donor **4** in CH_2Cl_2 was added into a pre-cooled solution of acceptor **5** and the promoter, trimethylsilyl trifluoromethanesulfonate (TMSOTf), in CH_2Cl_2 at −15 °C. As expected, synthesis of disaccharide **2** was successfully achieved in

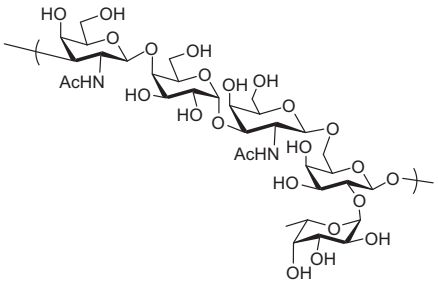

Figure 1. Structure of *E. coli* O128 antigen.

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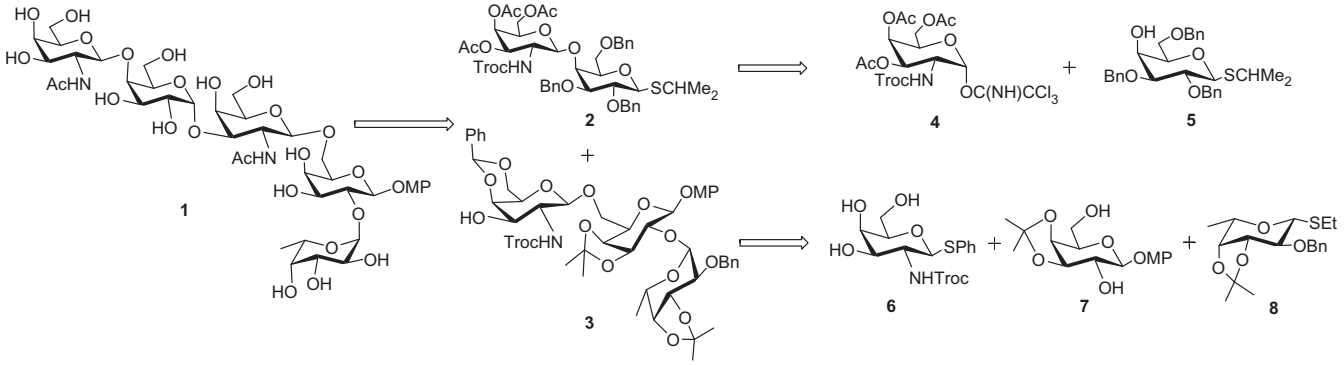
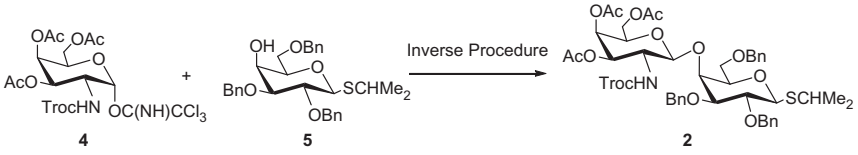
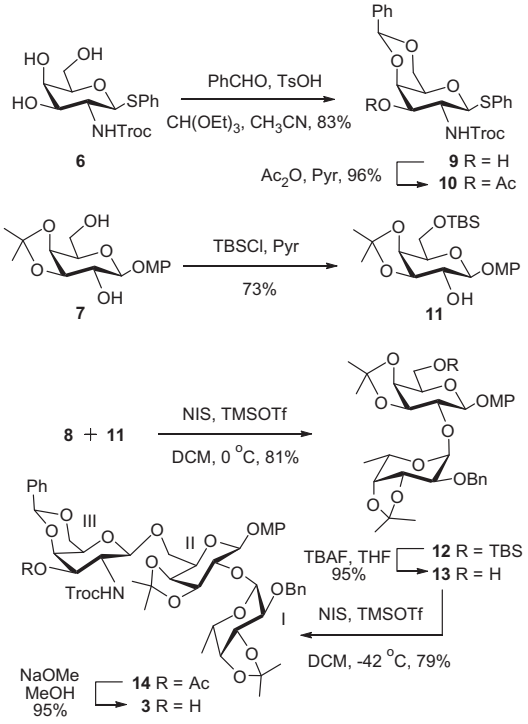
doi:10.1016/j.carres.2010.07.025



Scheme 1. Retrosynthetic analysis of *E. coli* O128 pentasaccharide.



Scheme 2. Synthesis of disaccharide donor **2**.



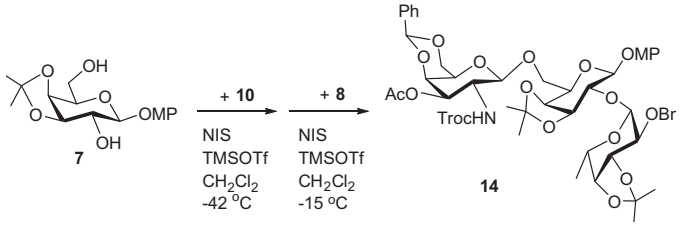
Scheme 3. Synthesis of trisaccharide acceptor **3**.

an acceptable isolated yield of 73%, in addition to its 6% of the α isomer.

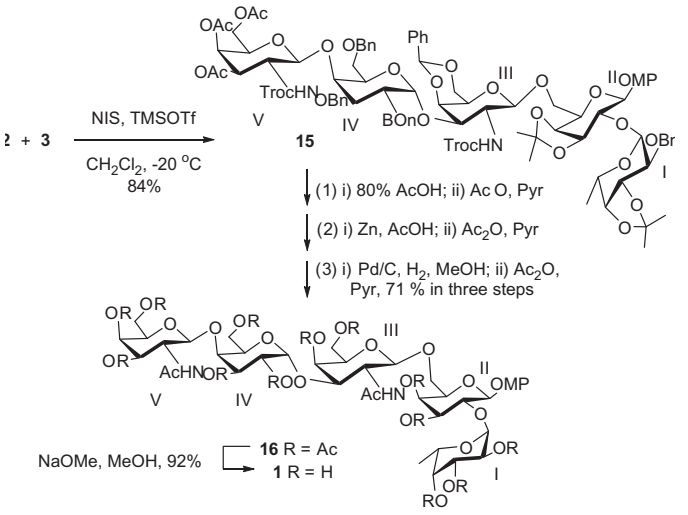
As depicted in Scheme 3, treatment of phenyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (**6**)[17] with PhCHO, trimethyl orthoformate [$CH(OEt)_3$], and TsOH in acetonitrile gave phenyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-1-thio-β-D-galactopyranoside (**9**), which was then acetylated with Ac_2O in pyridine to afford **10** in 79% yield over two steps. To differentiate the 2-OH from the 6-OH of 4-methoxyphenyl 3,4-*O*-isopropylidene-β-D-galactopyranoside (**7**), commercially available **7** was treated with TBSCl in pyridine at 0 °C, generating 4-methoxyphenyl 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (**11**). Condensation of ethyl 2-*O*-benzyl-3,4-*O*-isopropylidene-β-L-fucopyranoside (**8**)[18] and **11** in the presence of *N*-iodosuccinimide (NIS) and TMSOTf at 0 °C in CH_2Cl_2 gave disaccharide **12** (81%). Desilylation of **12** with tetrabutylammonium fluoride (TBAF) in THF yielded the disaccharide acceptor **13**, which was further coupled with **10**, under the same reaction conditions as described in the preparation of **12**, to afford trisaccharide **14**. Deacetylation of **14** with NaOMe in MeOH furnished trisaccharide acceptor **3** in a yield of 70% over three steps.

In order to improve the efficiency in making trisaccharide derivative **14**, we also investigated one-pot glycosylation as shown in Scheme 4. Taking advantage of different reactivity between the primary and secondary alcohols of acceptor **7**, donor **10** was first added to a pre-cooled (−42 °C) solution of **7** in CH_2Cl_2 in the presence of co-catalysts NIS–TMSOTf. An additional amount of **10** was added on occasion to push the reaction to completion. When acceptor **7** was completely consumed, the second glycosyl donor **8** was added to the same reaction system at −15 °C, together with another portion of NIS–TMSOTf. Compound **14** was eventually obtained in 28–43% isolated yields. However, this method provided unreliable yield, and the purification step was also tedious. We thus prepared **14** step-by-step as shown in Scheme 3.

With both disaccharide donor and trisaccharide acceptor in hands, we next explored the condensation of **2** and **3** catalyzed by NIS and TMSOTf in anhyd CH_2Cl_2 at −20 °C (Scheme 5). As we expected, pentasaccharide **15** was formed with the α-glycoside as the major product in 84% isolated yield. Based on COSY spectra of **15**, the chemical shifts corresponding to the new glycosidic bond showed at 5.25 ppm (^{1}H NMR, H-1^{IV}, J = 3.0 Hz) and 95.45 ppm



Scheme 4. One-pot synthesis of trisaccharide **14**.



Scheme 5. Synthesis of pentasaccharide **1**.

(^{13}C NMR, C-1^{IV}), respectively. Deacetalation of **15** with 80% AcOH at 85 °C, followed by removal of Troc with zinc in AcOH, debenzylation with 10% Pd/C under H_2 pressure, and acetylation of the residue with Ac_2O in pyridine, generated fully acetylated pentasaccharide **16** in 71% yield from **15**.[19] In the COSY spectra of **16**, a broad singlet at 5.13 ppm (J <3.0 Hz) and the peak at 94.6 ppm represent H-1^{IV} and C-1^{IV}, respectively, further confirming its structural assignment. Saponification of **16** with NaOMe in MeOH quantitatively furnished the desired pentasaccharide **1**.

In summary, we have developed a practical route for the synthesis of the pentasaccharide repeating unit of *E. coli* O128 antigen via a facile α-bond formation. This successful example supports the argument that remote controls affect the stereoselectivity in the construction of an oligosaccharide, leading to a new glycosyl bond with an opposite configuration to that of the established bond in either the donor or acceptor.[20] Application of this pentasaccharide in vaccine development is currently under exploration.

1. Experimental

1.1. 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 COSY spectra were recorded with Bruker ARX 500 (or 400) spectrometers for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. MALDI-TOF mass spectra (MALDI-TOFMS) were measured using α-cyano-4-hydroxycinnamic acid (CCA) as the matrix. 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 UV detection. Column chromatography was conducted by elution of a silica gel column (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent, or a column of Bio-Gel P2 with water as the eluent. Solutions were concentrated at <50 °C under reduced pressure.

1.2. Isopropyl 3,4,6-tri-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (2)

To a mixture of **5** (508 mg, 1.0 mmol) and 4 Å molecular sieves in anhyd CH_2Cl_2 (5 mL) was added TMSOTf (22 μL, 0.12 mmol) at −42 °C. The mixture was stirred under these conditions for 10 min with N_2 protection, and then a solution of **4** (750 mg, 1.2 mmol) in CH_2Cl_2 (10 mL) was added drop-by-drop. The reaction was monitored by TLC until compound **5** disappeared. The mixture was neutralized with Et_3N, and filtered, and the filtrate was concentrated. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave compound **2** (709 mg, 73%) as a syrup: $[\alpha]_D^{25}$ +86 (c 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26–7.42 (m, 15H, Ph), 5.62 (d, 1H, J 5.2 Hz, N*H*), 5.26 (d, 1H, J 2.4 Hz, H-4′), 4.92 (d, 1H, J 8.8 Hz, H-1), 4.88–4.91 (m, 2H), 4.79 (d, 1H, J 7.6 Hz, H-1′), 4.49–4.69 (m, 6H), 4.40 (d, 1H, J 11.5 Hz), 4.00–4.11 (m, 3H), 4.92–4.94 (m, 1H), 3.56–3.77 (m, 6H), 3.22–3.24 (m, 1H, SC*H*), 2.14, 1.99, 1.93 (3s, 9H, 3Ac), 0.85–0.88 (m, 6H, CH(CH_3)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.3, 170.1, 170.0, 154.1, 138.1, 137.1, 129.0, 128.7, 128.4, 128.2, 127.9, 127.7, 127.6, 101.9, 95.7, 84.7, 83.5, 78.9, 77.3, 77.0, 76.9, 76.7, 76.0, 75.7, 74.4, 74.3, 73.5, 71.8, 70.8, 69.2, 66.5, 61.1, 52.6, 35.3, 23.9, 23.8, 22.6, 20.6, 20.5. Anal. Calcd for $C_{45}H_{54}Cl_3NO_{14}S$: C, 55.64; H, 5.60. Found: C, 55.49; H, 5.50.

1.3. Phenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranoside (10)

A mixture of **6** (4.5 g, 10.0 mmol), PhCHO (1.2 mL, 12.0 mmol), CH(OEt)$_3$ (2.0 mL, 12.0 mmol), and TsOH (190 mg, 1.0 mmol) in CH_3CN (50 mL) was stirred at 45 °C for 5 h, at the end of which time TLC indicated that most of compound **6** was consumed. The reaction mixture was neutralized with Et_3N and concentrated under diminished pressure, and the residue was purified by column chromatography (3:1 petroleum ether–EtOAc) to give **9** as a foamy solid (4.47 g, 83%). A solution of **9** (4.27 g, 7.98 mmol) in Pyr (15 mL) and Ac_2O (8 mL) was stirred at rt for 6 h, then concentrated. Column chromatography (4:1 petroleum ether–EtOAc) of the residue gave **10** (4.42 g, 96%) as an amorphous solid: $[\alpha]_D^{25}$ −53 (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.25–7.66 (m, 10H, Ph), 5.55 (s, 1H, PhC*H*), 5.31 (d, 1H, J 10.2 Hz, H-3), 5.11 (d, 1H, J 8.0 Hz, N*H*), 5.07 (d, 1H, J 10.0 Hz, H-1), 4.73–4.75 (m, 2H, CH_2CCl$_3$), 4.37 (d, 1H, J 12.3 Hz, H-6a), 4.35 (s, 1H, H-4), 4.03 (d, 1H, J 12.3 Hz, H-6b), 3.90–3.93 (m, 1H, H-2), 3.60 (s, 1H, H-5), 2.03 (s, 3H, Ac). Anal. Calcd for $C_{24}H_{24}Cl_3NO_7S$: C, 49.97; H, 4.19. Found: C, 49.81; H, 4.25.

1.4. 4-Methoxyphenyl 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (11)

To a commercially available compound **7** (3.27 g, 10.0 mmol) in pyridine (25 mL) was added TBSCl (1.7 g, 12.0 mmol) at 0 °C. After stirring under these conditions for 3 h, the reaction was quenched by MeOH, and the mixture was concentrated. The residue was diluted with dichloromethane (50 mL), washed with satd aq $NaHCO_3$, and the organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (4:1 petroleum ether–EtOAc) to give **11** (3.21 g, 73%) as a syrup: $[\alpha]_D^{25}$ −28 (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.00, 6.79 (2d, 2 × 2H, J 8.0 Hz, $CH_3OC_6H_4O$), 4.63 (d, 1H, J 8.2 Hz, H-1), 4.22 (d, 1H, J 5.4 Hz, H-4), 4.13 (t, 1H, J 6.6 Hz, H-3), 4.88–4.90 (m, 3H, H-2, H-6a, H-6b), 3.80 (t, 1H, J 7.7 Hz, H-5), 3.75 (s, 3H, OCH_3), 2.76 (br s, 1H, O*H*), 1.55, 1.35 (2s, 6H, 2CH_3), 0.89, 0.06, 0.05 (3s, 15H, 5CH_3). Anal. Calcd for $C_{22}H_{36}O_7Si$: C, 59.97; H, 8.24. Found: C, 60.21; H, 8.17.

1.5. 4-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→2)-6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-β-D-galactopyranoside (12)

To a mixture of compounds **8** (406 mg, 1.2 mmol) and **11** (480 mg, 1.09 mmol) in anhyd CH_2Cl_2 (10 mL) were added NIS (405 mg, 1.8 mmol) and TMSOTf (22 μL, 0.12 mmol) under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 30 min, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that all the starting materials were consumed. The reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2

(10 mL), and washed with aq $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated. Column chromatography (6:1 petroleum ether–EtOAc) of the residue gave disaccharide **12** (633 mg, 81%) as a foamy solid: $[\alpha]_D^{25}$ −81 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.42 (m, 5H, Ph), 6.92, 6.80 (2d, 2 × 2H, *J* 9.1 Hz, CH_3O-C_6H_4O), 5.49 (d, 1H, *J* 3.6 Hz, H-1), 4.82 (d, 1H, *J* 8.2 Hz, H-1′), 4.78 (dd, 2H, PhCH_2), 4.58–4.60 (m, 1H), 4.34 (t, 1H, *J* 6.6 Hz, H-3′), 4.20–4.22 (m, 2H), 4.00–4.02 (m, 2H), 3.86–3.89 (m, 3H), 3.76 (s, 3H, CH_3OPh), 3.53 (dd, 1H, *J* 3.6, 8.0 Hz, H-2), 1.57 (s, 3H, H-6), 1.33–1.56 (2s, 12H, 4CH_3), 0.92 (s, 9H, C(CH_3)$_3$), 0.07 (2s, 6H, 2CH_3 of TBS). Anal. Calcd for $C_{38}H_{56}O_{11}Si$: C, 63.66; H, 7.87. Found: C, 63.49; H, 7.96.

1.6. 4-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→3)-3,4-*O*-isopropylidene-β-D-galactopyranoside (13)

A solution of **12** (700 mg, 0.98 mmol) and TBAF (523 mg, 2.0 mmol) in THF (15 mL) was stirred at 0 °C for 1 h, then for a further 4 h at rt until the starting material was consumed. The reaction mixture was diluted with EtOAc (10 mL), then washed with aq NH_4Cl and water, respectively. The water phase was extracted with EtOAc (3 × 3 mL), and the combined organic phase was washed with water and brine, dried, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to give **13** (560 mg, 95%) as a foamy solid: $[\alpha]_D^{25}$ −70 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.42 (m, 5H, Ph), 6.89, 6.82 (2d, 2 × 2H, *J* 9.1 Hz, $CH_3OC_6H_4O$), 5.47 (d, 1H, *J* 3.6 Hz, H-1), 4.89 (d, 1H, *J* 8.2 Hz, H-1′), 4.75 (dd, 2H, PhCH_2), 4.54–4.56 (m, 1H), 4.38 (t, 1H, *J* 6.1 Hz, H-3′), 4.24 (dd, 1H, *J* 5.6, 8.0 Hz, H-2′), 4.24 (dd, 1H, *J* 1.6, 5.6 Hz, H-4), 4.00–4.02 (m, 4H), 3.92 (br s, 1H, O*H*), 3.84 (s, 3H, CH_3OPh), 3.53 (dd, 1H, *J* 3.6, 8.0 Hz, H-2), 2.17 (s, 3H, CH_3), 1.34–1.38 (m, 12H, H-6 and 3CH_3). Anal. Calcd for $C_{32}H_{42}O_{11}$: C, 63.77; H, 7.02. Found: C, 63.60; H, 7.11.

1.7. 4-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→2)-[3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→6)]-3,4-*O*-isopropylidene-β-D-galactopyranoside (14)

To a mixture of compounds **10** (750 mg, 1.3 mmol) and **13** (602 mg, 1.0 mmol) in anhyd CH_2Cl_2 (12 mL) were added NIS (450 mg, 2.0 mmol) and TMSOTf (24 μL, 0.13 mmol) under an N_2 atmosphere at −42 °C. The mixture was stirred under these conditions for 1.5 h, quenched by Et_3N, diluted with CH_2Cl_2, and washed with aq $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give compound **14** (845 mg, 79%) as a white foamy solid: $[\alpha]_D^{25}$ −27 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24–7.52 (m, 10H, Ph), 6.96, 6.89 (2d, 2 × 2H, *J* 9.1 Hz, $CH_3OC_6H_4O$), 5.51 (s, 1H, C*H*Ph), 5.45 (d, 1H, *J* 3.6 Hz, H-1^I), 4.92 (d, 1H, *J* 8.4 Hz, H-1^{II}), 4.85–4.88 (m, 1H), 4.78 (dd, 2H, PhCH_2), 4.50 (d, 1H, *J* 10.0 Hz, H-1^{III}), 4.52–4.55 (m, 3H), 4.22–4.35 (m, 3H), 3.93–4.10 (m, 4H), 3.75 (s, 3H, CH_3OPh), 3.51–3.53 (m, 1H), 3.41 (s, 1H), 2.06 (s, 3H, Ac), 1.56, 1.38, 1.36, 1.34, 1.33 (5s, 15H, H-6^I, 4CH_3). Anal. Calcd for $C_{50}H_{60}Cl_{l3}NO_{18}$: C, 56.16; H, 5.66. Found: C, 56.32; H, 5.41.

1.8. 4-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→2)-[4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→6)]-3,4-*O*-isopropylidene-β-D-galactopyranoside (3)

NaOMe (1 M) was added to a solution of compound **14** (107 mg, 0.1 mmol) in MeOH (3 mL) at 0 °C until the pH of the solution reached 9–10. The reaction mixture was then stirred at rt for 4 h and neutralized with Dowex-50 (H^+) ion-exchange resin. The mixture was filtered, the filtrate was concentrated, and the resulting residue was purified by silica gel column chromatography (3:1 petroleum ether–EtOAc) to afford compound **3** (98 mg, 95%) as an amorphous solid: $[\alpha]_D^{25}$ −51 (*c* 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.25–7.50 (m, 10H, Ph), 6.97, 6.89 (2d, 2 × 2H, 9.0 Hz, $CH_3OC_6H_4O$), 5.54 (s, 1H, C*H*Ph), 5.45 (d, 1H, *J* 3.5 Hz, H-1^I), 4.91 (d, 1H, *J* 8.0 Hz, H-1^{II}), 4.78 (dd, 2H, PhCH_2), 4.67–4.69 (m, 2H), 4.24–4.35 (m, 3H), 3.93–4.12 (m, 9H), 3.75 (s, 3H, CH_3OPh), 3.51–3.53 (m, 1H), 3.41 (s, 1H), 1.56, 1.38, 1.36, 1.34, 1.33 (5s, 15H, H-6^I, 4CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.5, 154.9, 150.8, 138.1, 137.3, 129.2, 128.3, 128.2, 127.9, 127.6, 126.3, 118.1, 114.9, 110.6, 108.7, 101.3, 100.8, 99.6, 95.5, 79.8, 77.3, 77.0, 76.7, 76.2, 75.9, 75.7, 75.6, 74.9, 74.5, 73.9, 73.6, 71.8, 70.9, 69.0, 68.2, 66.5, 62.8, 55.6, 55.3, 28.2, 27.9, 26.5, 26.4, 16.3. Anal. Calcd for $C_{48}H_{58}Cl_3NO_{17}$: C, 56.12; H, 5.69. Found: C, 56.34; H, 5.58.

1.9. 4-Methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-galactopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-galactopyranosyl-(1→6)-[2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→2)]-3,4-*O*-isopropylidene-β-D-galactopyranoside (15)

To a mixture of **2** (233 mg, 0.24 mmol) and **3** (205 mg, 0.2 mmol) in anhyd CH_2Cl_2 (10 mL) were added NIS (81 mg, 0.36 mmol) and TMSOTf (4.0 μL, 0.022 mmol) under an N_2 atmosphere at −20 °C. The mixture was stirred under these conditions for 1.5 h, quenched by the addition of Et_3N, diluted with CH_2Cl_2 (20 mL), and washed with aq $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (1.5:1 petroleum ether–EtOAc) to give compound **15** (322 mg, 84%) as a white foamy solid: $[\alpha]_D^{25}$ +20 (*c* 2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.02–7.50 (m, 25H, Ph), 6.86–6.89 (m, 4H, $CH_3OC_6H_4O$), 5.52 (s, 1H, PhC*H*), 5.48 (d, 1H, *J* 3.2 Hz, H-1^I), 5.43 (d, 1H, *J* 7.4 Hz, N*H*), 5.25 (d, 1H, *J* 3.0 Hz, H-1^{IV}), 5.12 (s, 1H, H-4^V), 4.88 (d, 2H, *J* 11.0 Hz, PhCH_2), 4.78–4.81 (m, 3H), 4.67 (d, 1H, *J* 12.2 Hz, PhCH_2), 4.46–4.58 (m, 8H), 4.33 (t, 1H, *J* 6.3 Hz, H-3^{II}), 3.90–4.29 (m, 21H), 3.64–3.73 (m, 6H), 3.62–3.64 (m, 1H), 3.54 (dd, 1H, *J* 3.3, 8.1 Hz, H-5^I), 3.14 (s, 1H), 2.14, 2.03, 1.91 (3s, 9H, 3Ac), 1.59 (s, 3H), 1.37 (2s, 12H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.9, 173.2, 172.9, 170.0, 155.0, 154.4, 153.8, 151.4, 138.2, 137.9, 137.6, 128.9, 128.6, 128.2, 127.9, 127.6, 126.4, 117.5, 114.8, 110.4, 108.7, 102.0, 101.2, 101.1, 99.4,95.4, 80.0, 78.2, 77.3, 77.0, 76.7, 76.5, 76.3, 76.0, 75.7, 74.6, 74.1, 73.8, 72.5, 71.8, 71.2, 71.0, 70.4, 69.2, 68.8, 68.5, 66.6, 66.3, 62.8, 62.1, 61.4, 60.4, 55.6, 52.5, 39.3, 37.4, 37.1, 35.9, 34.0, 33.7, 33.4, 32.7, 31.9, 30.0, 29.7, 29.3, 29.1, 28.2, 28.0, 27.4, 27.2, 26.5, 24.8, 24.4, 22.7, 20.7, 19.7, 19.2, 18.3, 16.3, 14.4, 14.1. Anal. Calcd for $C_{90}H_{104}Cl_6N_2O_{31}$: C, 56.23; H, 5.45. Found: C, 56.02; H, 5.53.

1.10. 4-Methoxyphenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-α-D-galactopyranosyl-(1→3)-2-acetamido-4,6-di-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→6)-[2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl-(1→2)]-3,4-di-*O*-acetyl-β-D-galactopyranoside (16)

Compound **15** (401 mg, 0.21 mmol) in 80% aq AcOH (15 mL) was stirred at 85 °C for 35 min, at the end of which time the mixture was concentrated, and the residue was treated with Ac_2O (1 mL) in pyridine (2 mL) at rt for 4 h. The mixture was concentrated, and the residue was purified by flash silica gel column chromatography. The white foamy product thus generated was then dissolved in HOAc (12 mL), and zinc (nano-size activated powder, 99.9%, 800 mg) was added. After stirring for 6 h at 30 °C, TLC

(20:1 EtOAc–MeOH) showed that the starting material was completely consumed. The reaction mixture was passed through a short silica gel column (eluent: ethyl acetate), the combined eluent was concentrated, and the residue was again acetylated with Ac_2O in pyridine. The acetylated product was then dissolved in MeOH containing Pd/C (10% Pd, 50 mg), and H_2 was bubbled into the mixture at rt for about 12 h. The reaction mixture was filtered, and the filtrate was concentrated to dryness. Treatment of the residue with Ac_2O in pyridine as described above gave a syrup that was subjected to silica gel column chromatography (1:1 petroleum ether–EtOAc) to afford **16** (230 mg, 71%) as a foamy solid: $[\alpha]_D^{25}$ +17 (*c* 2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.97, 6.89 (2d, 2 × 2H, *J* 9.0 Hz, $CH_3OC_6H_4O$), 5.99 (d, 1H, *J* 7.5 Hz), 5.80 (dd, 1H, *J* 3.5, 13.5 Hz), 5.69 (d, 1H, *J* 7.5 Hz), 5.46 (d, 1H, *J* 3.5 Hz), 5.42 (dd, 1H, *J* 3.5, 11.0 Hz), 5.38 (d, 1H, *J* 3.5 Hz), 5.36 (d, 1H, *J* 4.0 Hz), 5.31 (dd, 1H, *J* 3.0, 11.0 Hz), 5.30 (d, 1H, *J* 4.0 Hz), 5.25 (d, 1H, *J* 3.0 Hz), 5.01–5.14 (m, 7H), 4.56–4.58 (m, 1H), 4.38–4.41 (m, 2H), 4.18–4.21 (m, 2H), 4.03–4.15 (m, 7H), 3.98 (t, 1H, *J* 8.5 Hz), 3.90 (t, 1H, *J* 7.0 Hz), 3.83–3.86 (m, 1H), 3.72–3.80 (m, 5H), 3.42–3.47 (m, 2H), 1.88–2.16 (m, 45H, 15Ac), 1.89 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2, 171.0, 170.6, 170.5, 170.4, 170.3, 170.2, 170.1, 170.0, 169.8, 155.5, 150.4, 117.6, 114.9, 99.6, 99.2, 99.1, 95.8, 94.6, 77.3, 77.0, 76.7, 73.8, 73.6, 72.4, 71.7, 71.5, 71.1, 70.7, 69.7, 68.3, 68.2, 67.4, 67.3, 66.7, 66.4, 66.2, 65.3, 64.7, 63.0, 61.7, 61.3, 55.7, 53.3, 23.3, 23.1, 21.0, 20.7, 20.6, 20.5, 15.8, 14.1. MALDI-TOFMS calcd for $C_{67}H_{90}N_2O_{39}$: 1456.5 $[M]^+$; found, 1569.7 $[M+Na]^+$. Anal. Calcd for $C_{67}H_{90}N_2O_{39}$: C, 52.00; H, 5.86. Found: C, 52.17; H, 5.96.

1.11. 4-Methoxyphenyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-α-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→6)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranoside (1)

To a solution of **16** (232 mg, 0.15 mmol) in MeOH (10 mL) was added 1 M NaOMe until the pH of the solution reached 9–10. The reaction mixture was allowed to stir at rt for 10 h, then neutralized with Dowex-50 (H^+) ion exchange resin. The mixture was filtered, the filtrate was concentrated, and the resulting residue was purified by a Bio-Gel P2 column using H_2O as eluent. The desired fractions were combined and freeze dried to afford compound **1** (138 mg, 92%) as an amorphous solid: $[\alpha]_D^{25}$ +5 (*c* 0.4, H_2O); ^1H NMR (500 MHz, D_2O): δ 7.08, 6.99 (2d, 2 × 2H, *J* 9.0 Hz, $CH_3OC_6H_4O$), 5.27 (s, 1H, H-1^I), 5.19 (d, 1H, *J* 7.5 Hz, H-1^{II}), 5.06 (d, 1H, *J* 4.0 Hz, H-1^{IV}), 4.59 (d, 1H, *J* 8.5 Hz, H-1^{III}), 4.53 (d, 1H, *J* 8.5 Hz, H-1^V), 3.58–4.21 (m, 33H), 2.05, 1.75 (2s, 6H, 2Ac), 1.11 (s, 3H, H-6^I). ^{13}C NMR (125 MHz, D_2O): δ 175.8, 174.9, 155.2, 151.5, 118.6, 118.1, 116.0, 103.7, 101.8, 100.7, 100.0, 96.3, 78.0, 77.5, 76.7, 75.6, 75.5, 74.5, 74.0, 72.5, 71.6, 71.3, 70.2, 69.9, 69.6, 69.2, 69.1, 69.0, 68.5, 67.7, 64.8, 61.8, 61.7, 60.8, 56.6, 53.3, 51.3, 23.1, 22.7, 20.8, 16.0. MALDI-TOFMS calcd for $C_{41}H_{64}N_2O_{26}$: 1000.4 $[M]^+$; found, 1023.7 $[M+Na]^+$. Anal. Calcd for $C_{41}H_{64}N_2O_{26}$: C, 49.20; H, 6.44. Found: C, 49.01; H, 6.52.

Acknowledgments

This work was supported by NNSF projects of China (20621703, 20872172, 20732001) and Project 2009ZX09501-011.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2010.07.025.

References

1. Orskov, F.; Orskov, I. *Methods Microbiolol.* **1984**, *14*, 43–112.
2. Raetz, C. R.; Whitefield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635–700.
3. Nataro, J. P.; Kaper, J. B. *Clin. Microbiol. Rev.* **1998**, *11*, 142–201.
4. Sengupta, P.; Bhattacharyya, T.; Shashkov, A. S.; Kochanowski, H.; Basu, S. *Carbohydr. Res.* **1995**, *277*, 283–290.
5. Kaiser, J. *Science* **2004**, *305*, 460.
6. Turnbull, J. E.; Field, R. A. *Nat. Chem. Biol.* **2007**, *3*, 74–77.
7. Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446*, 1046–1051.
8. Li, M.; Liu, X.; Shao, J.; Shen, J.; Jia, Q.; Yi, W.; Song, J. K.; Woodward, R.; Chow, C. S.; Wang, P. G. *Biochemistry* **2008**, *47*, 378–387.
9. Yang, F.; He, H.; Du, Y.; Lu, M. *Carbohydr. Res.* **2002**, *337*, 1165–1169.
10. Kanaya, T.; Yagi, S.; Schweizer, F.; Takeda, T.; Kiuchi, F.; Hada, N. *Chem. Pharm. Bull.* **2010**, *58*, 811–817.
11. Cheng, L.; Chen, Q.; Liu, J.; Du, Y. *Carbohydr. Res.* **2007**, *342*, 975–981.
12. Hanessian, S. *Preparative Carbohydrate Chemistry*; Marcel Dekker: New York, 1997.
13. Kihlberg, J.; Eichler, E.; Bundle, D. R. *Carbohydr. Res.* **1991**, *211*, 59–75.
14. Knapp, S.; Nandan, S. R. *J. Org. Chem.* **1994**, *59*, 281–283.
15. Leigh, D. A.; Smart, J. P.; Truscello, A. M. *Carbohydr. Res.* **1995**, *276*, 417–424.
16. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353–3356.
17. Imamura, A.; Ando, H.; Ishida, H.; Kiso, M. *Org. Lett.* **2005**, *7*, 4415–4418.
18. Smid, P.; de Ruiter, G. A.; van der Marel, G. A.; Rombouts, F. M.; van Boom, J. H. *J. Carbohydr. Chem.* **1991**, *10*, 833–849.
19. Greene, T. W.; Wuts, P. G. M. *Protecting Groups in Organic Synthesis*; Wiley: New York, 1999; 510–511.
20. Zeng, Y.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 2047–2056.