Carbohydrate Research 391 (2014) 43–47

Contents lists available at ScienceDirect

Carbohydrate Research

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

Synthesis of *Calocybe indica* var. APK2 polysaccharide repeating unit

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ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 1 April 2014
Accepted 2 April 2014
Available online 12 April 2014

Keywords:
Mushroom polysaccharide
Repeating unit
Synthesis
Glycosidation

ABSTRACT

The first total synthesis of *p*-methoxyphenyl α-L-fucopyranosyl-(1→6)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**2**) was achieved starting from five monosaccharide building blocks. This structure represents the repeating unit of the polysaccharide isolated from edible mushroom *Calocybe indica* var. APK2, and was synthesized in high overall yield via a convergent '3+2' glycosylation strategy.

1. Introduction

A water-soluble polysaccharide was isolated recently from a hot aqueous extract of fruit bodies of edible mushroom *Calocybe indica* var. APK2.[1] The primary structure of the repeating unit of this polysaccharide was identified as →3)-[α-L-Fuc*p*-(1→6)]-α-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→6)-β-D-Glc*p*-(1→, that is, the pentasaccharide structure **1** depicted in Figure 1. The subsequent biological assay showed that this polysaccharide could inhibit effectively human cervical cancer HeLa cell growth with IC_{50} being 1 μg/mL, whereas no significant growth inhibition was found up to 100 μg/mL against normal cells.[1] Furthermore, this mushroom-derived polysaccharide exhibited strong immunostimulatory activity by stimulating proliferation of splenocytes, thymocytes and bone marrow cells;[1] it may thus find use in immunotherapy as well.

In view of the interesting anticancer and immunostimulatory activities, investigations toward the synthesis of the pentasaccharide repeating unit of the polysaccharide that may prove useful in biological studies were initiated. We wish to report here a convergent synthesis of the *para*-methoxyphenyl (MP) glycoside of pentasaccharide **2** as part of our research program to identify potent immunostimulants.[2] The reducing end MP group could be readily removed on demand, and would also facilitate the purification and characterization of the final product.

2. Results and discussion

The target pentasaccharide is relatively simple in structure, but it contains two α-glycosidic linkages that are considered to be synthetic challenges in carbohydrate chemistry.[3] Our retrosynthetic analysis revealed a convergent [3+2] approach to be attractive (Scheme 1). Target structure **2** may be obtained from fully protected pentasaccharide **3** through a three-step deprotection sequence, including hydrolysis of the isopropylidene group, hydrogenolysis, and cleavage of the ester groups. In turn, pentasaccharide **3** will be assembled from trisaccharide donor **4** and disaccharide acceptor **5**. Trisaccharide **4** can be derived from thiofucoside **6**,[4] MP galactoside **7**,[5] and thioglucoside **8**.[6] The benzyl protecting group at the 2-*O*-position of **6** and **7** will favor the formation of the corresponding α-glycosidic linkages by virtue of kinetic anomeric effect. This effect was well elaborated in a recent commentary article.[7] Disaccharide **5** can be accessed from thioglucoside **9**[8] and MP glucoside **10**.[9] The 2-hydroxyl groups of **8** and **9** are protected with benzoyl and acetyl groups, respectively, with a view to make use of neighboring group participation[10] to ensure the formation of the corresponding β-glycosidic bonds.

All the monosaccharide building blocks **6**–**10** that are needed for the synthesis of pentasaccharide **2** are known compounds and can be readily accessed following literature procedures. The assembly of the pentasaccharide started with the coupling of donor **6** and acceptor **7**. Thiofucoside **6** and galactoside **7** were dissolved in CH_2Cl_2, and the mixture was treated with *N*-iodosuccinimide (NIS)[11] and trimethylsilyl triflate (TMSOTf) at −15 °C to produce the desired disaccharide **11** in 85% yield (Scheme 2). Structural assignments of **11** were made on the basis of NMR spectroscopy

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http://dx.doi.org/10.1016/j.carres.2014.04.004
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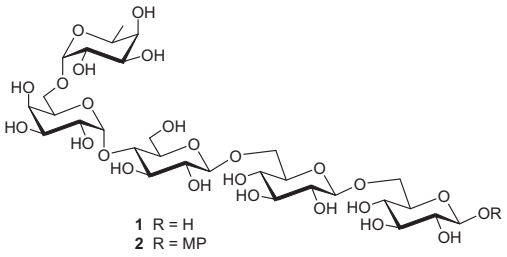
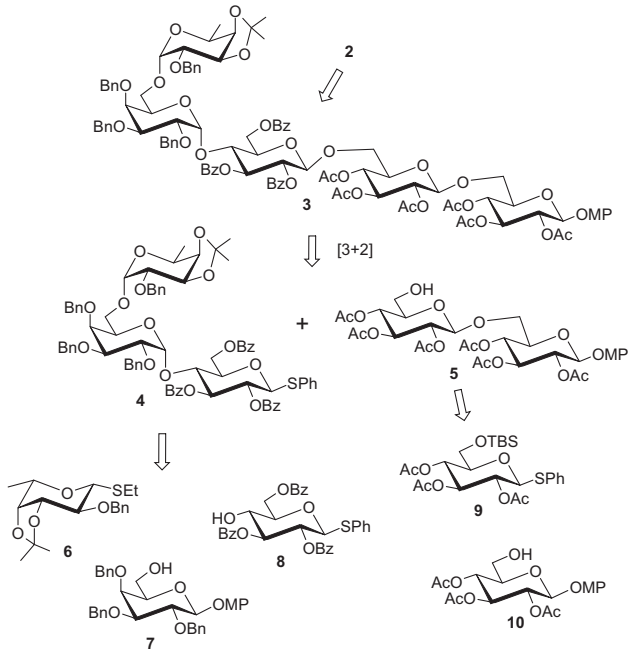
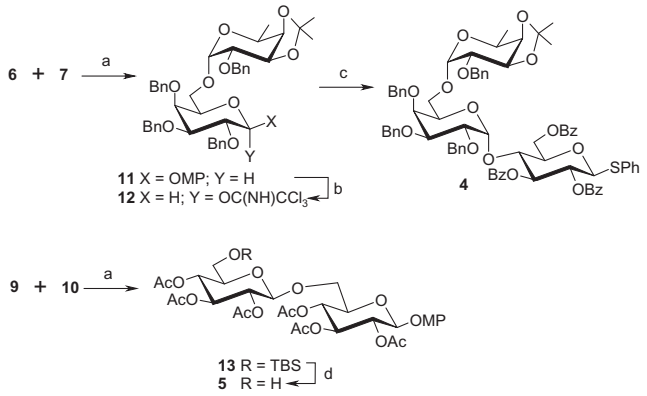


Figure 1. Structures of target pentasaccharide **1** and its MP glycoside **2**.



Scheme 1. Retrosynthetic analysis of pentasaccharide **2**.



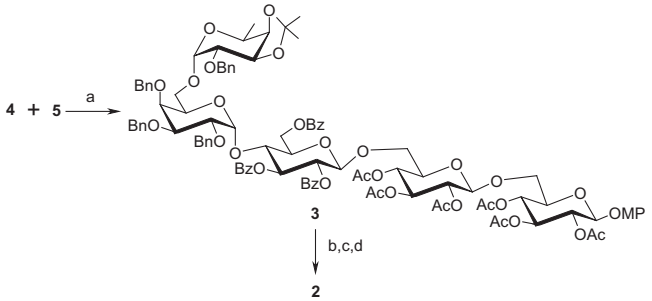
Scheme 2. Synthesis of trisaccharide **4** and disaccharide **5**. Reagents and conditions: (a) NIS, TMSOTf, CH_2Cl_2, −15 °C, 85% for **11**, 86% for **13**; (b) (i) CAN, CH_3CN, H_2O; (ii) CCl_3CN, DBU, CH_2Cl_2, 90% (two steps); (c) **8**, TMSOTf, CH_2Cl_2, inverse procedure, −15 °C, 84%; and (d) TBAF, HOAc, THF, 85%.

and exact mass spectral data. The α configuration of the newly formed glycosidic bond was readily determined by the coupling constant $^3J_{H1-H2}$ value, which is about 3.6 Hz. Subsequently, **11** was converted into the corresponding trichloroacetimidate **12** in two steps by deprotection with ceric ammonium nitrate (CAN) followed by imidation with CCl_3CN. The coupling of imidate **12** with acceptor **8** was then performed under normal Schmidt conditions[12] to synthesize trisaccharide donor **4**, but unfortunately, a significant amount of byproduct formed by transfer of the phenylthio group from **8** to **12** was isolated from the reaction. Aglycon transfer of thioglycosides has been reported numerous times in the past decades.[13] When a glycosyl acceptor possessing a thioglycoside aglycon is reacted with a glycosyl donor, the sulfide group can be transferred from the acceptor to the donor. This is relatively common side reaction in glycosidation reactions involving thioglycoside as acceptor. We attributed this side reaction to the poor nucleophilicity of the 4-OH group of acceptor **8**, which rendered glycosylation of anomeric thio group possible. To avoid the side reaction, the inverse procedure[14] that might boost the nucleophilicity of acceptor was applied to the glycosidation reaction. Hence, acceptor **8** was premixed with catalytic amounts of TMSOTf in CH_2Cl_2, and imidate **12** was then added slowly to the resulting solution. As expected, this time the desired trisaccharide **4** was produced smoothly as indicated by TLC and was isolated in 84% yield. The anomeric stereochemistry of the galactose residue in **4** was confirmed by the chemical shift of the anomeric proton and the coupling constant $^3J_{H1-H2}$ value: 4.94 ppm and $^3J_{H1-H2}$ = 3.5 Hz. The ^{13}C chemical shift of the anomeric carbon of the galactosidic bond was also in the expected region for α anomer (97.6 ppm).

Meanwhile, synthesis of the disaccharide fragment **5** was also carried out as shown in Scheme 2. Glycosylation of pre-prepared acceptor **10** with thioglycoside donor **9** under the action of NIS/TMSOTf smoothly generated the desired disaccharide **13** in very high yield (86%), which was then subjected to standard desilylation conditions (TBAF/HOAc/THF) to give **5** in 85% yield.

Having trisaccharide donor **4** and disaccharide acceptor **5** in hand, attention was then focused on their coupling to assemble the target molecule. Fortunately, activation of **4** with NIS/TMSOTf in the presence of **5** led successfully to the desired pentasaccharide **3** in 86% yield (Scheme 3). Clearly, the complete β-stereoselectivity in this glycosidation resulted from the assistance of the participating benzoyl group at the C-2 position of glucose moiety in donor **4**. Final deprotection of **3** to convert into the target molecule **2** was achieved in three steps, as shown in Scheme 3: firstly, hydrolysis of the isopropylidene group with 80% HOAc followed by acetylation; secondly, debenzylation by catalytic hydrogenation in the presence of $Pd(OH)_2/C$; finally, cleavage of the ester groups under Zemplén conditions. The target molecule **2** was produced in 76% yield over three steps. It was purified by size exclusion chromatography on Bio-Gel P2 (eluant: H_2O), and characterized by NMR and HR-ESIMS.

In summary, the first total synthesis of the repeating pentasaccharide unit of a polysaccharide isolated from edible mushroom *C. indica* var. APK2 was achieved in this report. An efficient [3+2]



Scheme 3. Synthesis of target molecule **2**. Reagents and conditions: (a) NIS, TMSOTf, CH_2Cl_2, −25 °C, 86%; (b) (i) 80% HOAc; (ii) Ac_2O, Py; (c) $Pd(OH)_2/C$, EtOAc, MeOH; and (d) NaOMe, MeOH, 76% (three steps).

convergent strategy was applied to the synthesis, and all the glycosidic bonds were introduced in highly stereoselective mode. In view of the important bioactivities of the mushroom polysaccharide, especially the immunostimulatory activity, the synthesized pentasaccharide is of great interest for studying its biological properties. Work is in progress to test the immunological bioactivity of the synthesized sample.

3. Experimental

3.1. General methods

Reactions were performed in oven-dried glassware. Solvents were evaporated under reduced pressure while maintaining the water bath temperature below 50 °C. All reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} and the compounds visualized by UV or by treatment with 8% H_2SO_4 in methanol followed by heating. Flash chromatography was performed with the appropriate solvent system using 100–200 mesh silica. Optical rotations were measured at 20 °C with a Perkin–Elmer 241-MC polarimeter (1 dm cell). ^{1}H NMR spectra were obtained on a 400 or 600 MHz and reported in parts per million (δ) relative to the response of the solvent or to tetramethylsilane (0.00 ppm). Coupling constants (J) are reported in Hertz (Hz). ^{13}C NMR spectra were recorded at 100 or 150 MHz and reported in δ relative to the response of the solvent. Yields refer to chromatographically pure compounds and are calculated based on reagents consumed.

3.2. *p*-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (11)

To a mixture of **6** (0.76 g, 2.25 mmol) and **7** (1.44 g, 1.88 mmol) in CH_2Cl_2 (14 mL) were added NIS (0.84 g, 3.75 mmol) and TMSOTf (40 μL, 0.22 mmol) under N_2 atmosphere at −15 °C. After stirring at this temperature for 1.5 h, the reaction mixture was quenched with Et_3N (50 μL) and diluted with CH_2Cl_2 (20 mL). The mixture was then washed with aqueous $Na_2S_2O_3$ (1 M, 10 mL), the organic layer was dried over Na_2SO_4 and concentrated in vacuo to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 3:1) to give **11** (1.34 g, 85%) as a syrup: $[\alpha]_D$ −36.6 (*c* 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.21 (m, 19H), 7.22 (m, 1H), 7.05 (d, *J* = 8.9 Hz, 2H), 6.77 (d, *J* = 8.9 Hz, 2H), 5.08 (d, *J* = 11.7 Hz, 1H), 5.01 (d, *J* = 10.8 Hz, 1H), 4.85 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 7.7 Hz, 1H), 4.76 (d, *J* = 11.8 Hz, 1H), 4.74 (d, *J* = 3.6 Hz, 1H), 4.69 (t, *J* = 11.8 Hz, 2H), 4.61 (d, *J* = 12.9 Hz, 2H), 4.22 (dd, *J* = 5.5, 7.7 Hz, 1H), 4.08 (dd, *J* = 7.8, 9.6 Hz, 1H), 3.90 (d, *J* = 2.6 Hz, 1H), 3.88 (dd, *J* = 1.8, 6.6 Hz, 1H), 3.86 (dd, *J* = 2.4, 5.5 Hz, 1H), 3.70 (s, 3H), 3.68 (d, *J* = 2.1 Hz, 1H), 3.66 (s, 1H), 3.65 (d, *J* = 5.7 Hz, 1H), 3.58 (dd, *J* = 2.7, 9.8 Hz, 1H), 3.45 (dd, *J* = 3.3, 7.7 Hz, 1H), 1.37 (s, 3H), 1.32 (s, 3H), 1.13 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 155.4, 151.7, 138.6, 138.5, 138.4, 138.3, 128.4, 128.3, 128.2, 127.9, 127.8, 127.6, 127.5, 119.2, 114.4, 108.6, 103.6, 97.9, 82.2, 79.3, 76.2, 76.0, 75.7, 75.5, 74.4, 73.7, 73.2, 72.2, 67.2, 63.3, 55.6, 28.1, 26.3, 16.2; ESI-HRMS calcd for $C_{50}H_{56}NaO_{11}$ $[M+Na]^+$ 855.3829, found 855.3823.

3.3. 2-*O*-Benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl trichloroacetimidate (12)

To a solution of **11** (1.27 g, 1.53 mmol) in CH_3CN (20 mL) and H_2O (5 mL) was added ammonium cerium nitrate (1.36 g, 2.48 mmol) at room temperature. After stirring for 1.5 h, the reaction mixture was diluted with EtOAc and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to give a yellowish amorphous solid. To a solution of the above solid (1.00 g, 1.37 mmol) in CH_2Cl_2 (10 mL) were added trichloroacetonitrile (0.41 mL, 4.10 mmol) and DBU (41 μL) at 0 °C. After stirring for 3 h, the reaction mixture was concentrated, and the residue was purified by flash column chromatography (petroleum ether/EtOAc, 3:1) to give **12** (1.07 g, 90%) as an amorphous white solid: $[\alpha]_D$ −46 (*c* 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (s, 1H), 7.40–7.29 (m, 20H), 6.61 (d, *J* = 3.4 Hz, 1H), 5.15 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 6.8 Hz, 1H), 4.80 (br s, 3H), 4.77 (d, *J* = 12.3 Hz, 1H), 4.74 (d, *J* = 11.8 Hz, 1H), 4.71 (d, *J* = 12.4 Hz, 1H), 4.63 (d, *J* = 11.5 Hz, 1H), 4.31 (dd, *J* = 3.4, 10.0 Hz, 1H), 4.28–4.24 (m, 2H), 4.16 (br s, 1H), 4.08 (dd, *J* = 2.6, 10.1 Hz, 1H), 4.00 (dd, *J* = 2.4, 6.6 Hz, 1H), 3.95 (dd, *J* = 2.4, 5.5 Hz, 1H), 3.81 (dd, *J* = 7.9, 10.5 Hz, 1H), 3.61 (dd, *J* = 5.8, 10.5 Hz, 1H), 3.53 (dd, *J* = 3.4, 7.7 Hz, 1H), 1.44 (s, 3H), 1.38 (s, 3H), 1.21 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.3, 138.7, 138.5, 138.4, 138.3, 128.4, 128.3, 128.2, 127.8, 127.6, 127.5, 127.4, 108.8, 97.5, 95.3, 91.5, 78.0, 76.0, 75.8, 75.7, 73.1, 73.0, 65.8, 63.4, 28.1, 26.3, 16.2; ESI-HRMS calcd for $C_{45}H_{50}Cl_3NNaO_{10}$ $[M+Na]^+$ 892.2535, found 892.2539.

3.4. Phenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-1-thio-β-D-glucopyranoside (4)

A suspension of **8** (168 mg, 0.29 mmol), TMSOTf (5.7 μL, 44 μmol), and 4 Å molecular sieves in CH_2Cl_2 (5 mL) was stirred at −15 °C for 20 min under nitrogen, and a solution of **12** (275 mg, 0.32 mmol) in CH_2Cl_2 (3 mL) was added dropwise to the suspension. The resulting mixture was stirred until TLC indicated complete consumption of acceptor **8**, then quenched with Et_3N (60 μL), filtered. The filtrate was concentrated in vacuo to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to yield the title compound **4** (312 mg, 84%) as an amorphous white solid: $[\alpha]_D$ −6.8 (*c* 0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.1 Hz, 2H), 7.94 (d, *J* = 7.1 Hz, 2H), 7.91 (d, *J* = 7.1 Hz, 2H), 7.67 (t, *J* = 7.8 Hz, 1H), 7.47–7.02 (m, 33H), 5.85 (t, *J* = 9.3 Hz, 1H), 5.36 (t, *J* = 9.7 Hz, 1H), 5.02 (dd, *J* = 1.9, 12.2 Hz, 1H), 5.00 (d, *J* = 3.8 Hz, 1H), 4.92 (t, *J* = 10.9 Hz, 2H), 4.96 (d, *J* = 3.5 Hz, 1H), 4.74 (s, 2H), 4.54 (s, 2H), 4.51 (dd, *J* = 6.0, 12.1 Hz, 1H), 4.44 (d, *J* = 11.5 Hz, 1H), 4.24–4.21 (m, 2H), 4.11 (dd, *J* = 6.0, 7.2 Hz, 1H), 4.07 (t, *J* = 9.6 Hz, 1H), 3.97 (d, *J* = 12.6 Hz, 1H), 3.94–3.92 (m, 2H), 3.91 (dd, *J* = 2.1, 6.5 Hz, 1H), 3.88–3.85 (m, 2H), 3.80 (dd, *J* = 3.5, 10.2 Hz, 1H), 3.60 (dd, *J* = 7.2, 10.2 Hz, 1H), 3.48 (dd, *J* = 3.5, 7.7 Hz, 1H), 3.45 (dd, *J* = 6.5, 10.2 Hz, 1H), 1.36 (s, 3H), 1.28 (s, 3H), 1.15 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.0, 165.5, 165.3, 138.6, 138.5, 138.4, 138.2, 133.2, 133.0, 132.8, 132.2, 129.9, 129.8, 129.6, 129.3, 128.8, 128.5, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.5, 127.4, 108.8, 100.0, 97.6, 85.8, 78.8, 77.5, 76.0, 75.9, 75.8, 75.6, 75.1, 74.9, 74.5, 73.1, 73.0, 71.8, 70.8, 70.4, 66.8, 64.0, 63.2, 28.1, 26.3, 16.2; ESI-HRMS calcd for $C_{76}H_{76}NaO_{17}S$ $[M+Na]^+$ 1315.4865, found 1315.4869.

3.5. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (13)

To a solution of **9** (1.18 g, 2.31 mmol) and **10** (0.91 g, 2.20 mmol) in CH_2Cl_2 (20 mL) were added NIS (1.04 g, 4.40 mmol) and TMSOTf (39.8 μL, 0.26 mmol) under N_2 atmosphere at −15 °C. The mixture was stirred at this temperature for 1 h, then quenched with Et_3N (50 μL), diluted with CH_2Cl_2 (20 mL), and washed with

aqueous $Na_2S_2O_3$ (1 M, 12 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 3:2) to give the disaccharide **13** (1.54 g, 86%) as a white foam: $[\alpha]_D$ +2.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.91 (d, *J* = 4.5 Hz, 2H), 6.85 (d, *J* = 9.1 Hz, 2H), 5.23 (t, *J* = 7.8 Hz, 1H), 5.17 (dd, *J* = 7.8, 9.6 Hz, 1H), 5.12 (t, *J* = 9.5 Hz, 1H), 5.02 (t, *J* = 9.5 Hz, 1H), 4.94–4.13 (m, 3H), 4.55 (d, *J* = 7.9 Hz, 1H), 3.85 (dd, *J* = 1.8, 11.3 Hz, 1H), 3.80 (dd, *J* = 1.8, 9.6 Hz, 1H), 3.76 (s, 3H), 3.67–3.63 (m, 3H), 3.43–3.41 (m, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.84 (s, 3H), 0.85 (br s, 9H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 170.2, 169.5, 169.4, 169.3, 169.2, 155.7, 151.1, 129.0, 128.2, 118.1, 114.8, 100.3, 99.9, 74.6, 73.8, 73.2, 72.7, 71.3, 71.2, 68.8, 68.6, 67.7, 62.1, 55.6, 25.8, 20.7, 20.6, 20.5, 18.3, −5.3; ESI-HRMS calcd for $C_{37}H_{54}NaO_{18}Si$ $[M+Na]^+$ 837.3145, found 837.3149.

3.6. *p*-Methoxyphenyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (5)

Disaccharide **13** (3.09 g, 3.80 mmol) was dissolved in dry THF (40 mL) and cooled to 0 °C under N_2. HOAc (1.87 mL) was added dropwise followed by addition of a solution of TBAF in THF (1.0 M, 4.54 mL, 4.54 mmol). The cooling bath was removed and the reaction was allowed to warm to room temperature and stirred overnight. Brine was then added and the mixture was extracted several times with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 2:1) to give the title compound **5** (2.26 g, 85%) as a syrup: $[\alpha]_D$ −6.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, *J* = 6.0 Hz, 2H), 6.89 (d, *J* = 6.0 Hz, 2H), 5.28 (t, *J* = 6.3 Hz, 1H), 5.21 (dd, *J* = 6.3, 12.5 Hz, 2H), 5.03 (dd, *J* = 5.2, 11.6 Hz, 2H), 4.98 (d, *J* = 5.2 Hz, 2H), 4.64 (d, *J* = 5.3 Hz, 1H), 4.14 (dd, *J* = 4.7, 9.4 Hz, 1H), 3.91 (dd, *J* = 1.6, 7.4 Hz, 1H), 3.81 (s, 3H), 3.76–3.73 (m, 2H), 3.58 (dd, *J* = 3.2, 4.4 Hz, 1H), 3.45–3.43 (m, 1H), 2.54 (br s, 1H), 2.08–2.06 (m, 9H), 2.04 (s, 3H), 2.03 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 170.1, 169.9, 169.3, 169.2, 155.5, 150.7, 118.6, 114.4, 100.1, 98.8, 73.6, 72.6, 71.8, 71.1, 70.3, 69.6, 69.0, 68.2, 65.3, 61.7, 55.4, 25.7, 20.9, 20.8, 20.6, 20.5, 18.2, 14.1; ESI-HRMS calcd for $C_{31}H_{40}O_{18}Na$ $[M+Na]^+$ 723.2212, found 723.2198.

3.7. *p*-Methoxyphenyl 2-*O*-benzyl-3,4-*O*-isopropylidene-α-L-fucopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (3)

Donor **4** (243 mg, 0.19 mmol) and acceptor **5** (120 mg, 0.17 mmol) were dissolved in CH_2Cl_2 (5 mL) and cooled to −25 °C under N_2 atmosphere. NIS (77 mg, 0.34 mmol) was added to this solution, followed by addition of TMSOTf (3.1 μL, 17 μmol). The reaction mixture was stirred at this temperature for 1 h, then quenched with Et_3N (20 μL), diluted with CH_2Cl_2 (10 mL), and washed with aqueous $Na_2S_2O_3$ (1 M, 5 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc, 3:2) to give the pentasaccharide **3** (277 mg, 86%) as a white foam: $[\alpha]_D$ −14.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (d, *J* = 7.4 Hz, 2H), 7.95 (dd, *J* = 3.9, 7.4 Hz, 4H), 7.63–7.25 (m, 23H), 7.21 (d, *J* = 7.2 Hz, 2H), 7.17 (d, *J* = 7.4 Hz, 2H), 7.07 (d, *J* = 5.3 Hz, 2H), 6.95 (d, *J* = 9.0 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 5.88 (t, *J* = 9.4 Hz, 1H), 5.41 (dd, *J* = 7.8, 9.6 Hz, 1H), 5.25 (t, *J* = 9.5 Hz, 1H), 5.19 (t, *J* = 7.9 Hz, 1H), 5.10 (d, *J* = 3.4 Hz, 1H), 5.06 (t, *J* = 9.4 Hz, 1H), 5.00 (d, *J* = 11.0 Hz, 1H), 4.94 (d, *J* = 11.6 Hz, 1H), 4.92 (t, *J* = 7.6 Hz, 2H), 4.87 (t, *J* = 8.4 Hz, 1H), 4.82 (t, *J* = 10.8 Hz, 1H), 4.78 (dd, *J* = 3.6, 8.4 Hz, 2H), 4.75 (d, *J* = 4.7 Hz, 2H), 4.57 (s, 3H), 4.47 (d, *J* = 11.4 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.28 (d, *J* = 12.4 Hz, 1H), 4.25 (dd, *J* = 7.4, 9.4 Hz, 1H), 4.22 (d, *J* = 9.0 Hz, 1H), 4.14 (t, *J* = 6.6 Hz, 1H), 4.01 (d, *J* = 12.4 Hz, 1H), 3.97 (s, 1H), 3.94–3.92 (m, 2H), 3.91–3.87 (m, 2H), 3.85 (d, *J* = 3.6 Hz, 1H), 3.82 (dd, *J* = 3.6, 9.6 Hz, 1H), 3.78 (s, 3H), 3.72 (d, *J* = 11.4 Hz, 1H), 3.65–3.61 (m, 3H), 3.58 (t, *J* = 7.2 Hz, 1H), 3.50 (dd, *J* = 3.3, 7.7 Hz, 1H), 3.46 (dd, *J* = 6.3, 10.1 Hz, 1H), 3.39 (dd, *J* = 6.5, 11.4 Hz, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H), 1.38 (s, 3H), 1.31 (s, 3H), 1.16 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.7, 170.3, 170.1, 169.6, 169.5, 169.4, 166.0, 165.5, 165.2, 155.7, 151.1, 138.6, 138.5, 138.3, 133.3, 133.1, 133.0, 129.8, 129.7, 129.4, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.7, 127.4, 127.3, 118.4, 114.7, 108.8, 101.2, 100.2, 100.1, 100.0, 97.4, 78.8, 76.2, 76.0, 75.8, 75.7, 75.1, 74.9, 74.4, 74.2, 73.7, 73.2, 73.1, 72.9, 72.8, 72.0, 71.8, 70.2, 69.1, 68.7, 68.5, 67.7, 66.6, 63.7, 63.2, 55.6, 29.6, 28.1, 26.3, 20.7, 20.6, 20.5, 16.1; ESI-HRMS calcd for $C_{101}H_{110}NaO_{35}$ $[M+Na]^+$ 1905.6842, found 1905.6899.

3.8. *p*-Methoxyphenyl α-L-fucopyranosyl-(1→6)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (2)

A solution of pentasaccharide **3** (420 mg, 0.22 mmol) in 80% AcOH (20 mL) was stirred at 80 °C for 5 h and then concentrated in vacuo, and the traces of HOAc and water were removed by coevaporation several times with toluene. The residue was treated directly with Ac_2O (2 mL) and pyridine (4 mL), and stirred at room temperature for 5 h. The mixture was then concentrated, diluted with EtOAc, washed successively with 5% HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to give a white foam, which was directly used in the next step without purification. The foam was dissolved in MeOH/EtOAc (15 mL, 1:1 v/v). $Pd(OH)_2$/C (20%, 60 mg) was added and the suspension was stirred under H_2 atmosphere for 8 h, after which time the mixture was filtered, and the filtrate was concentrated in vacuo to afford the crude intermediate, the partially protected pentasaccharide. The crude material was then dissolved in MeOH (20 mL) and treated with a 1 M solution of NaOMe in MeOH until PH of the solution reached 9–10. The mixture was stirred at room temperature for 10 h and then neutralized with Amberlite IR-120 resin. The mixture was filtrated and concentrated, and the resulting residue was purified by size exclusion chromatography on a Bio-Gel P2 column (15 mm × 820 mm) using H_2O as eluent. After lyophilization with water, the target pentasaccharide **2** (134 mg, 76% over three steps) was obtained as an amorphous solid: $[\alpha]_D$ −142 (*c* 0.5, H_2O); H NMR (600 MHz, D_2O) δ 7.17 (d, *J* = 8.9 Hz, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 5.07 (d, *J* = 4.6 Hz, 1H), 4.97 (d, *J* = 3.3 Hz, 1H), 4.50 (d, *J* = 7.8 Hz, 2H), 4.44 (d, *J* = 7.9 Hz, 1H), 4.20 (t, *J* = 10.8 Hz, 2H), 4.13 (t, *J* = 1.7 Hz, 2H), 3.98 (s, 1H), 3.93–3.90 (m, 4H), 3.84–3.79 (m, 10H), 3.69 (dd, *J* = 2.2, 10.0 Hz, 1H), 3.65 (dd, *J* = 1.2, 6.0 Hz, 1H), 3.62 (d, *J* = 9.6 Hz, 1H), 3.58 (d, *J* = 7.9 Hz, 3H), 3.55 (d, *J* = 9.6 Hz, 2H), 3.50 (d, *J* = 4.2 Hz, 2H), 3.34 (d, *J* = 8.4 Hz, 1H), 3.31 (d, *J* = 9.1 Hz, 1H), 1.24 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (150 MHz, D_2O) δ 154.8, 150.8, 118.3, 115.1, 103.2, 102.8, 100.9, 99.3, 98.9, 83.1, 81.0, 79.3, 76.2, 75.5, 75.3, 74.5, 74.3, 73.7, 73.1, 72.9, 72.8, 72.6, 71.8, 70.7, 69.5, 68.6, 68.2, 67.4, 66.8, 60.1, 55.8, 15.3; ESI-HRMS calcd for $C_{37}H_{58}O_{26}Na$ $[M+Na]^+$ 941.3264, found: 941.3083.

Acknowledgements

This work was supported by the Natural Science Foundation of Zhejiang Province (R4110195), the Educational Commission of

Zhejiang Province (Y201328123) and the Foundation of State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (KF2012-17).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2014.04.004.

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