Carbohydrate Polymers 88 (2012) 185–193

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Chemo-enzymatic synthesis of xylogluco-oligosaccharides and their interactions with cellulose

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ARTICLE INFO

Article history:
Received 20 September 2011
Received in revised form
22 November 2011
Accepted 27 November 2011
Available online 23 December 2011

Keywords:
Xylogluco-oligosaccharide
Bacterial cellulose
Enzymatic synthesis
Cellulase
Cellosynthase

ABSTRACT

A novel class of complex oligosaccharides with a backbone made of twelve β-(1→4)-D-glucosyl units that may be regularly substituted on their primary position with α-D-xylosyl or β-D-(1→2)-galactosyl-α-D-xylosyl residues were readily synthesized using enzymatic hydrolysis of the plant polysaccharide xyloglucan (XG), followed by chemical modifications of the well-defined oligosaccharides and their enzymatic coupling catalyzed by the Cel7B E197A glycosynthase from *Humicola insolens*. These complex oligosaccharides proved useful for a better understanding of the effects of structural variation of xyloglucan on interactions with bacterial microcrystalline cellulose (BMCC).

1. Introduction

Primary cell walls of dicotyledons and non-graminaceous monocots are made of cellulose microfibrils embedded in a matrix of pectins, hemicelluloses and proteins (Hayashi, 1989; O'Neill & York, 2003; Somerville et al., 2004). XGs are the most abundant hemicellulose found in plant primary cell wall (Carpita & Gibeaut, 1993). XG is a complex heteropolysaccharide that is composed of a cellulose-like backbone of β-(1→4) linked D-Glc*p* residues that are regularly substituted at *O*-6 position with an α-D-Xyl*p* residue. An unambiguous XG nomenclature was developed where the repeating units of XGs are described from the non-reducing end to the reducing end with a single letter that denotes a specific glucosyl residue substitution pattern. An unsubstituted D-Glc*p* is assigned "G" and α-D-Xyl*p*-(1→6)-β-D-Glc*p* segment is assigned "X" (Fry et al., 1993). Xyloglucan from most plants is composed of XXXG repeating subunits, while XGs from plants in Poaceae and Solanaceae are composed of XXGG motifs (Vincken, York, Beldman, & Voragen, 1997). Further substitution occurs primarily on *O*-2 position at specific xylosyl residues with a variety of sugar moieties; the most common being β-D-Gal*p*, α-L-Ara*f* (only in Poaceae and Solanaceae) or the disaccharide α-L-Fuc*p*-(1→2)-β-D-Gal*p* (Carpita & Gibeaut, 1993). The β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p* unit is assigned "L", while α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p* is assigned "F" (Fry et al., 1993). The flour produced from seed kernel of *Tamarindus indica* contains about 50% of XGs made of four repetitive units: XXXG, XXLG, XLXG and XLLG in the ratio of 13:28:9:50 and in which no fucosyl unit was detected (York, Van Halbeek, Darvill, & Albersheim, 1990).

Since the beginning of the 90s the role of these substituents in the binding of XG onto cellulose microfibrils has been studied, but there are multiple variations in both the number and pattern of these substitutions, so to assess the specific role of only one factor on polymeric XGs is rather difficult and it is not completely elucidated. On the one hand, simulation of molecular conformation of xyloglucans by Levy, York, Stuike-Prill, Meyer and Staehelin (1991) suggested that fucosyl–galactosyl substitution of XGs determines a flat conformation to the polymer and is responsible for its capacity to bind to cellulose. This assumption was later on confirmed experimentally by Langmuir adsorption isotherms (Hayashi, Ogawa, & Mitsuishi, 1994; Levy, Maclachlan, & Staehelin, 1997). On the other hand, isothermal titration calorimetry experiments reported more recently, disproved this hypothesis (Lima, loh, & Buckeridge, 2004). Furthermore, *in vitro* studies using *Rubus fructicosus* XGs suggested that binding increases as xylosyl motifs decrease (Chambat, Karmous, Costes, Picard, & Joseleau, 2005). Later on, different models were used to study XG–cellulose interactions

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doi:10.1016/j.carbpol.2011.11.085

as a function of both cellulose and XG characteristics (Lopez et al., 2010). In particular, the impact of XG characteristics such as the molecular weight or the nature of side chains was studied using different sources of XGs and their corresponding xylogluco-oligosaccharides (XGOs) obtained after enzymatic hydrolysis. It was also confirmed that XGOs in which the backbone is made of twelve glucosyl units are the smallest entities that may interact with cellulose.

In this paper, a complementary approach is taken; we aimed to assess the putative role of unsubstituted zone of XGs on the composite formation with cellulose since it was reported that XGOs bind less efficiently than cello-oligosaccharides with similar glucosyl residues (Hayashi et al., 1994). The efficiency of the XGO motif XXXGGGGGXXXG is compared to fully substituted ones XXXGXXXGXXXG and XXLGXXLGXXLG. Thus, we report a strategy for synthesizing these XGOs that combines enzymatic hydrolysis of tamarind XGs, chemical modifications of the resulting XGOs and enzymatic couplings that involve the "cellosynthase" Cel7B E197A from *Humicola insolens* (Fort et al., 2000; Fort, Christiansen, Schulein, Cottaz, & Driguez, 2000).

A comparative study using adsorption isotherm determination was performed in order to evaluate the affinity of these XGOs with bacterial microcrystalline cellulose.

2. Experimental

2.1. General methods

β-Galactosidase from *Aspergillus niger* and cellulase 3042A were gifts, respectively, from Megazyme (Bray, Ireland) and Genencor (USA). Evolution of reactions was monitored by analytical thin-layer chromatography using silica gel 60 F254 precoated plates (E. Merck, Darmstadt). Compounds were purified by column chromatography using silica gel Si 60 (63–200 μm, E. Merck, Darmstadt) and subsequently filtered with a syringe-driven filter unit (0.45 μm; Millex®-HV) before lyophilization. Roman numerals in ascending order are given to the residues from the reducing end. The same Roman numeral is given to a glucosyl residue and its C-6 substituted xylosyl residue and its C-2 substituted galactosyl residues. NMR spectra were recorded at 298 K with a Bruker Avance 400 MHz spectrometer. Proton chemical shifts (δ) are reported in ppm with the residual HOD peak as an internal reference (δ_{HOD} = 4.77 ppm) (Gottlieb, Kotlyar, & Nudelman, 1997). Coupling constants (J) are in Hertz (Hz); multiplicities are singlet (s), doublet (d), and multiplet (m).

Mass spectrometry measurements. MALDI-TOF mass spectra were acquired at CERMAV (UPR CNRS 5301) using a time-of-flight mass spectrometer (Autoflex Serie 1, Bruker Daltonics) in reflectron positive ion mode (260 cm effective flight path; 20 kV). Voltages were IS1 19 kV and IS2 16 kV, and the PIE delay was 90 ns. A nitrogen laser was used for desorption/ionization (λ = 337 nm, 0.5 ns, pulse energy of 100 μJ). Time-of-flight spectra were generated by signal averaging of 10 deposits on sample holder with 240 laser shots (diameter < 150 μm) by deposit. Compounds were solubilized in water or MeOH (200 pmol/μL). The matrix solution was prepared by solubilizing DHB (2,5-dihydroxybenzoic acid, Sigma) in MeOH (50 mg/mL). For analysis, a mixture of 0.5 μL of sample solution and 0.5 μL of DHB solution was deposited on the sample holder and dried overnight at atmospheric pressure.

High-resolution electrospray mass spectra in the positive ion mode were obtained at Laboratoire des Glucides (UMR 6219 Université de Picardie Jules Vernes) on a Q-TOF Ultima Global hybrid quadrupole/time-of-flight instrument (Waters-Micromass, Manchester, U.K.), equipped with a pneumatically assisted electrospray (Z-spray) ion source and an additional sprayer (Lock Spray) for the reference compound. The source and desolvation temperatures were kept at 80 and 150 °C, respectively. Nitrogen was used as the drying and nebulizing gas at flow rates of 350 and 50 L/h, respectively. The capillary voltage was 3.5 kV, the cone voltage 100 V and the RF lens1 energy was optimised for each sample (40 V). For collision-induced dissociation (CID) experiments, argon was used as collision gas at an indicated analyzer pressure of 5.10^{-5} Torr and the collision energy was optimised for each parent ion (50–110 V). Lock mass correction, using appropriate cluster ions of sodium iodide $(NaI)_nNa^+$, was applied for accurate mass measurements. The mass range was typically 50–2050 Da and spectra were recorded at 2 s/scan in the profile mode at a resolution of 10,000 (FWMH). Data acquisition and processing were performed with MassLynx 4.0 software. Acetylated oligosaccharides were dissolved in MeOH and deprotected compounds in H_2O/MeOH.

2.2. Substrate preparation and characterizations

*2.2.1. [(2.3.4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2.3.4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2.3.4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-α,β-D-glucopyranose (**5**) per(OAc)-XLLG*

Tamarind seed xyloglucans (7.5 g) were suspended in water for 2 h at 60 °C. The mixture was cooled down to 37 °C and cellulase 3042A (1.35 mL) was added with sodium azide (100 mg). After incubation for 4 h at 37 °C, the solution was lyophilized. Residue was solubilized in pyridine (220 mL). Acetic anhydride (110 mL) and dimethylamino pyridine (110 mg) were added at 0 °C. Resulting solution was stirred overnight at room temperature (rt) in darkness and poured into ice-cold water (1 L). Precipitate was filtered through Celite®, dissolved in CH_2Cl_2 and dried over magnesium sulfate. Mixture was concentrated and co-evaporated with toluene (×3). Purification by flash chromatography on silica gel (1:2 toluene/acetone) afforded the title compound **5** (2.8 g, 58% mass yield). LRMS (MALDI): m/z = 2502 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 6.20 (d, $J_{1,2}$ = 3.7 Hz, 0.4H, H-$1^{Glc\,I\alpha}$); 5.64 (d, $J_{1,2}$ = 8.2 Hz, 0.6H, H-$1^{Glc\,I\beta}$); 5.44–5.31 (m, 6H, H-$3^{Glc\,I\alpha,\,IV,\,Xyl\,II,\,III,\,IV}$, H-$4^{Gal\,II,\,III}$); 5.24–5.09 (m, 6H, H-$2^{Gal\,II,\,III}$, H-$3^{Glc\,I\beta,\,II,\,III}$, H-$4^{Glc\,IV}$); 5.01–4.88 (m, 11H, H-$1^{Xyl\,II,\,III,\,IV}$, H-$2^{Glc\,I,\,II,\,III}$, H-$3^{Gal\,II,\,III}$, H-$4^{Xyl\,II,\,III,\,IV}$); 4.80–4.75 (m, 3H, H-$1^{Glc\,IV}$, H-2 $Glc^{Glc\,IV,\,Xyl\,IV}$) 4.68–4.51 (m, 5H, H-$1^{Glc\,II,\,III,\,Gal\,II,\,III}$, H-$6a^{Glc\,I}$); 4.15–4.06 (m, 5H, H-$6^{Gal\,II,\,III}$, H-$6b^{Glc\,I}$); 3.96–3.46 (m, 22H, H-$2^{Xyl\,II,\,III}$, H-$4^{Glc\,I,\,II,\,III}$, H-$5^{Glc\,I,\,III,\,IV,\,Xyl\,II,\,III,\,IV,\,Gal\,II,\,III}$, H-$6^{Glc\,II,\,III,\,IV}$); 3.43 (m, 1H, H-$5^{Glc\,II}$); 2.14–1.95 (m, 78H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–169.0 (CH_3CO); 101.6, 1001.5 (C-$1^{Gal\,II,\,III}$); 101.0, 100.7, 100.6 (C-$1^{Glc\,II,\,III,\,IV}$); 98.8, 98.7, 98.6 (C-$1^{Xyl\,II,\,III}$); 96.5 (C-$1^{Xyl\,IV}$); 91.8 (C-$1^{Glc\,I\beta}$); 89.2 (C-$1^{Glc\,I\alpha}$); 77.4, 76.0, 75.9, 75.4, 75.3, 74.6, 73.9, 73.8, 73.2, 73.1, 72.7, 72.5, 72.4, 72.2 (2C), 71.8 (2C), 71.4, 71.3 (2C), 70.9, 70.8, 70.7, 69.7, 69.6, 69.4 (2C), 69.1 (2C), 68.3 (2C), 67.8, 67.1 (2C), 66.5, 64.5 (C-$2^{Glc\,I,\,II,\,III,\,IV,\,Xyl\,II,\,III,\,IV,\,Gal\,II,\,III}$, C-$3^{Glc\,I,\,II,\,III,\,IV,\,Xyl\,II,\,III,\,IV,\,Gal\,II,\,III}$, C-$4^{Glc\,I,\,II,\,III,\,IV,\,Xyl\,II,\,III,\,IV,\,Gal\,II,\,III}$, C-$5^{Glc\,I,\,II,\,III,\,IV,\,Gal\,II,\,III}$, C-$6^{Glc\,II,\,III,\,IV}$); 61.7 (C-$6^{Glc\,I\alpha}$); 61.5 (C-$6^{Glc\,I\beta}$); 61.4, 61.3 (C-$6^{Gal\,II,\,III}$); 59.4 (2C); 58.9 (C-$5^{Xyl\,II,\,III,\,IV}$); 21.1–20.7 ($CH_3CO$). HRMS (ESI) calcd for $C_{103}H_{138}O_{69}Na^+$: 2501.7187, found: 2501.7173.

*2.2.2. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-α,β-D-glucopyranose (**6**)*
XLLG

Compound **5** (184 mg, 74 μmol) was de-*O*-acetylated by treatment with methanolic sodium methoxide at rt as previously described (Greffe, Bessueille, Bulone, & Brumer III, 2005). Nonasaccharide 6 was obtained with 95% yield. LRMS (MALDI): *m*/*z* = 1409 $[M+Na]^+$.

*2.2.3. [(2.3.4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2.3.4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2.3.4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α,β-D-glucopyranose (**7**)*
per(OAc)-XLLG-OH

Hydrazine acetate (58 mg, 0.63 mmol, 2.0 equiv.) was added to a solution of compound **5** (770 mg, 0.31 mmol, 1.0 equiv.) in DMF (9 mL) at 50 °C. The reaction mixture was stirred at 50 °C for 30 min and diluted in ethyl acetate (15 mL). The solution was washed with brine, dried over magnesium sulfate, concentrated *in vacuo*. The crude mixture was purified by flash chromatography on silica gel (3:2 toluene/acetone) afforded the title compound **7** (444 mg, 0.18 mmol, 58% yield). LRMS (MALDI): *m*/*z* = 2460 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 5.59 (t, *J* = 9.7 Hz, 0.6H, H-1$^{Glc\ I}$); 5.45–5.27 (m, 6H, H-3$^{Glc\ IV,\ Xyl\ II,\ III,\ IV}$, H-4$^{Gal\ II,\ III}$); 5.22–5.08 (m, 6H, H-2$^{Gal\ II,\ III}$, H-3$^{Glc\ I,\ II,\ III}$, H-4$^{Glc\ IV}$); 5.01–4.84 (m, 11H, H-1$^{Xyl\ II,\ III,\ IV}$, H-2$^{Glc\ I,\ II,\ III}$, H-3$^{Gal\ II,\ III}$, H-4$^{Xyl\ II,\ III,\ IV}$); 4.81–4.48 (m, 7H, H-1$^{Glc\ II,\ III,\ IV,\ Gal\ II,\ III}$, H-2 Glc$^{Glc\ IV,\ Xyl\ IV}$); 4.49 (dd, $J_{5,6a}$ = 1.5 Hz and $J_{6a,6b}$ = 11.0 Hz, 1H, H-6a$^{Glc\ I}$); 4.27–4.07 (m, 5H, H-6$^{Gal\ II,\ III}$, H-6b$^{Glc\ I}$); 3.89–3.52 (m, 23H, H-2$^{Xyl\ II,\ III}$, H-4$^{Glc\ I,\ II,\ III}$, H-5$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, H-6$^{Glc\ II,\ III,\ IV}$); 2.09–1.95 (m, 78H, C*H*$_3$CO); 1.79 (br, 1H, O*H*). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–169.0 (CH_3CO); 101.7, 101.6 (C-1$^{Gal\ II,\ III}$); 100.9, 100.6 (2C) (C-1$^{Glc\ II,\ III,\ IV}$); 98.7, 98.6 (C-1$^{Xyl\ II,\ III}$); 96.4 (C-1$^{Xyl\ IV}$); 90.3 (C-1$^{Glc\ I}$); 76.0, 75.9, 75.7, 75.4, 74.9, 74.8, 74.7, 73.7, 73.1, 72.9, 72.6, 72.4, 72.1 (2C), 72.0, 71.9, 71.8, 71.6, 71.4, 71.3, 70.9, 70.8 (2C), 69.4, 69.4 (3C), 69.2, 69.1, 68.6, 68.5, 68.3, 68.2, (C-2$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-3$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-4$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-5$^{Glc\ I,\ II,\ III,\ IV,\ Gal\ II,\ III}$); 67.7 (C-6$^{Glc\ IV}$); 67.2 (C-6$^{Glc\ III}$); 67.1 (C-6$^{Glc\ II}$); 62.1 (C-6$^{Glc\ I\alpha}$); 61.4 (2C) (C-6$^{Gal\ II,\ III}$); 61.3 (C-6$^{Glc\ I\beta}$); 59.4, 58.9 (2C) (C-5$^{Xyl\ II,\ III,\ IV}$); 21.2–20.7 ($CH_3CO$). HRMS (ESI) calcd for $C_{101}H_{136}O_{68}Na^+$: 2459.7082, found: 2459.7144.

*2.2.4. [(2,3,4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl fluoride (**8**) per(OAc)-XLLG-αF*

Diethylaminosulfur trifluoride (DAST) (120 μL, 902 μmol, 5.0 equiv.) was added to a solution of compound **7** (442 mg, 180 μmol, 1.0 equiv.) in CH_2Cl_2 (8 mL) previously cooled down to −30 °C. The mixture was stirred for 1 h at −30 °C, allowed to warm up at rt and stirred overnight. DAST was quenched by addition of MeOH (1 mL) before concentration. Purification by flash chromatography on silica gel (1:3 petroleum ether/ethyl acetate) afforded the fluorinated compound as a mixture of anomers. The mixture was treated with a solution of HF/pyridine (7/3, 5 mL) added at −50 °C to the solid residue in a plastic container. The solution was stirred and allowed to warm up at −10 °C for 2.5 h. After dilution with CH_2Cl_2 (5 mL), the solution was slowly poured in ice-cold ammonia (3 M). The solution was extracted with CH_2Cl_2 (×2), organic layers was washed with $NaHCO_{3sat}$, combined and concentrated. Purification by flash chromatography on silica gel previously neutralized with triethylamine (1:2 toluene/ethyl acetate) afforded the title compound **8** (357 mg, 146 μmol, 81% yield).

LRMS (MALDI): *m*/*z* = 2461 $[M+Na]^+$. $[\alpha]_D$ = +40 (*c* = 1.0, chloroform). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 5.62 (dd, $J_{1,2}$ = 2.4 Hz, $J_{H,F}$ = 53.1 Hz; 1H, H-1$^{Glc\ I}$); 5.46–5.33 (m, 6H, H-3$^{Glc\ I\alpha,\ IV,\ Xyl\ II,\ III,\ IV}$, H-4$^{Gal\ II,\ III}$); 5.25–5.09 (m, 6H, H-2$^{Gal\ II,\ III}$, H-3$^{Glc\ I\beta,\ II,\ III}$, H-4$^{Glc\ IV}$); 5.05–4.90 (m, 10H, H-1$^{Xyl\ II,\ III,\ IV}$, H-2$^{Glc\ I,\ III}$, H-3$^{Gal\ II,\ III}$, H-4$^{Xyl\ II,\ III,\ IV}$); 4.86 (m, 1H, H-2$^{Glc\ II}$); 4.80–4.77 (m, 3H, H-1$^{Glc\ IV}$, H-2 Glc$^{Glc\ IV,\ Xyl\ IV}$); 4.71 (d, $J_{1,2}$ = 8,5 Hz, 1H, H-1$^{Glc\ III}$); 4.61–4.55 (m, 4H, H-1$^{Glc\ II,\ Gal\ II,\ III}$, H-6a$^{Glc\ I}$); 4.17–4.03 (m, 5H, H-6$^{Gal\ II,\ III}$, H-6b$^{Glc\ I}$); 3.97–3.62 (m, 21H, H-2$^{Xyl\ II,\ III}$, H-4$^{Glc\ I,\ II,\ III}$, H-5$^{Glc\ I,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, H-6$^{Glc\ II,\ III}$, H-6a$^{Glc\ IV}$); 3.56 (dd, $J_{5,6b}$ = 3.9 Hz and $J_{6a,6b}$ = 12.1 Hz, 1H, H-6b$^{Glc\ IV}$); 3.47 (m, 1H, H-5$^{Glc\ II}$); 2.15–1.95 (m, 75H, C*H*$_3$CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–169.4 (CH_3CO); 103.0 (d, $J_{C,F}$ = 226.0 Hz, C-1$^{Glc\ I}$); 101.5, 101.4 (C-1$^{Gal\ II,\ III}$); 100.9, 100.7, 100.6 (C-1$^{Glc\ II,\ III,\ IV}$); 98.9, 98.8 (C-1$^{Xyl\ II,\ III}$); 96.4 (C-1$^{Xyl\ IV}$); 77.4, 76.1, 75.8, 75.2, 75.0, 74.5, 74.3, 73.8, 73.2, 73.1, 72.6, 72.4, 72.1 (2C); 71.7 (2C); 71.4, 71.3, 70.9, 70.8 (2C); 70.7, 69.8, 69.4, 69.3 (2C); 69.2 (2C); 68.9, 68.3 (2C); 67.9, 67.1 (2C); 66.6, 66.4 (C-2$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-3$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-4$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-5$^{Glc\ I,\ II,\ III,\ IV,\ Gal\ II,\ III}$, C-6$^{Glc\ II,\ III,\ IV}$); 61.4, 61.3 (2C) (C-6$^{Glc\ I,\ Gal\ II,\ III}$); 59.4 (2C), 58.9 (C-5$^{Xyl\ II,\ III,\ IV}$); 21.2–20.3 ($CH_3CO$). HRMS (ESI) calcd for $C_{101}H_{135}O_{67}FNa^+$: 2461.7038, found: 2461.7021.

*2.2.5. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl fluoride (**9**)*
XLLG-αF

Sodium methoxide (1 M, 60 μL, 1.7 equiv., final pH 12) was added at 0 °C to a solution of compound **8** (89 mg, 36 μmol, 1.0 equiv.) in methanol (10 mL). The solution was allowed to warm up to rt and stirred overnight, neutralized with Amberlite IR120-H^+, filtered, concentrated and co-evaporated twice with H_2O to remove MeOH. Lyophilization afforded the title compound **9** (47 mg, 34 μmol, 93% yield). LRMS (MALDI): *m*/*z* = 1412 $[M+Na]^+$. HRMS (ESI) calcd for $C_{51}H_{85}O_{42}FNa^+$: 1411.4397, found: 1411.4430.

*2.2.6. [(2,3,4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-1,2,3,6-tetra-O-acetyl-α,β-D-glucopyranose (**10**)*
per(OAc)-XXLG

Tamarind xyloglucans (10.5 g) were hydrolyzed by cellulase 3042A as previously described for the preparation of compound **5**. Medium was acidified at pH 4–5 by acetic acid (50%) and β-D-galactosidase from *A. niger* (10 μL, 0.4 mg) was added. Incubation was maintained 48 h at 25 °C under gentle stirring, before addition of another aliquot of enzyme (18 μL, 0.6 mg). After an additional incubation of 48 h at 25 °C, the reaction mixture was boiled to neutralize the enzyme and lyophilized. The mixture of XGOs obtained was acetylated in acetic anhydride/pyridine (1/2 (v/v), 450 mL). After one night at room temperature in the dark, medium was precipitated in ice-water (2 L). Crude

product was obtained after filtration followed by dissolution in dichloromethane and evaporation of the solvent. Purification by flash chromatography on silica gel (3:2 toluene/acetone) afforded the per(OAc)-XXLG **10** (45% yield). LRMS (MALDI): m/z = 2214 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 6.19 (d, $J_{1,2}$ = 3.7 Hz, 0.4H, H-1$^{Glc\ I\alpha}$); 5.65 (d, $J_{1,2}$ = 8.2 Hz, 0.6H, H-1$^{Glc\ I\beta}$); 5.44–5.30 (m, 4H, H-3$^{Glc\ I\alpha,\ IV,\ Xyl\ II,\ III,\ IV}$); 5.23–4.82 (m, 14H); 4.80–4.63 (m, 6H); 4.59–4.46 (m, 3H); 4.15–4.12 (m, 2H); 4.12–3.61 (m, 22H); 3.44 (m, 1H, H-5$^{Glc\ II}$); 2.08–1.95 (m, 69H, CH_3CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–169.5 (CH_3CO); 101.7, 101.6 (2s, C-1$^{Glc\ II}$); 100.8, 100.7, 100.6, 100.5 (4s, C-1$^{Glc\ III,\ IV,\ Gal\ II}$); 98.4, 98.3, 97.5 (3s, C-1$^{Xyl\ II,\ III}$); 96.1 (C-1$^{Xyl\ IV}$); 91.7 (C-1$^{Glc\ I\beta}$); 89.2 (C-1$^{Glc\ I\alpha}$); 75.9, 75.8, 75.7, 75.6, 75.3, 75.0, 74.4, 74.2, 73.9, 73.8, 73.4, 73.3, 72.7, 72.5, 72.4, 72.1, 72.0, 71.9, 71.5, 71.1, 70.9, 70.8, 70.7, 70.5, 69.7, 69.6, 69.4, 69.3, 69.2 (C-2$^{Glc\ I,II,III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-3$^{Glc\ I,II,III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-4$^{Glc\ I,II,III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-5$^{Glc\ I,II,III,\ IV,\ Gal\ II}$); 68.4, 68.3, 67.6, 67.1, 65.6, 65.5 (C-6$^{Glc\ II,\ III,\ IV}$); 61.4 (C-6$^{Gal\ II}$); 61.9 (C-6$^{Glc\ I\alpha}$); 61.6 (C-6$^{Glc\ I\beta}$); 59.3 (2C), 59.0 (C-5$^{Xyl\ II,\ III,\ IV}$); 21.2–20.6 (*C*H_3CO). HRMS (ESI) calcd for $C_{91}H_{122}O_{61}Na^+$: 2213.6342, found: 2213.3287.

*2.2.7. [(2,3,4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α,β-D-glucopyranose (**11**)*
per(OAc)-XXLG-OH

The title compound **11** was obtained from compound **10** as described for the preparation of compound **7**. Purification by flash chromatography on silica gel (5:4 cyclohexane/acetone) afforded the title compound **11** (99% yield). LRMS (MALDI): m/z = 2172 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 5.57–5.28 (m, 5H, H-1$^{Glc\ I}$, H-3$^{Glc\ IV,\ Xyl\ II,\ III,\ IV}$); 5.40 (t, J = 9.3 Hz, 1H, H-3$^{Glc\ I}$); 5.37–5.28 (m, 4H, H-3$^{Glc\ IV,\ Xyl\ II,\ III,\ IV}$); 5.20–4.87 (m, 13H, H-1$^{Xyl\ II,\ III,\ IV}$, H-2$^{Glc\ I,\ II,\ Gal\ II}$, H-3$^{Glc\ II,\ III}$, H-4$^{Glc\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$); 4.84–4.66 (m, 8H, H-1$^{Glc\ III,\ IV,\ Gal\ II}$, H-2$^{Glc\ III,\ IV,\ Xyl\ III,\ IV}$, H-3$^{Gal\ II}$); 4.56 (d, J = 8.1 Hz, 1H, H-1$^{Glc\ II}$); 4.52 (dd, $J_{5,6a}$ = 1.9 Hz and $J_{6a,6b}$ = 11.9 Hz, 1H, H6a$^{Glc\ I}$); 4.23–4.05 (m, 5H, H-5$^{Glc\ I}$, H-6$^{Gal\ II}$, H-6a$^{Glc\ III}$, H-6b$^{Glc\ I}$); 4.02–3.74 (m, 13H, H-2$^{Xyl\ II}$, H-4$^{Glc\ I,\ II,\ III}$, H-5$^{Glc\ IV,\ Xyl\ III,\ Gal\ II}$, H-5a$^{Xyl\ II}$, H-6$^{Glc\ II}$, H-6a$^{Glc\ IV}$, H-6b$^{Glc\ III}$); 3.70–3.60 (m, 5H, H-5$^{Glc\ III,\ Xyl\ IV}$, H-5b$^{Xyl\ II}$, H-6b$^{Glc\ IV}$); 3.48 (m, 1H, H-5$^{Glc\ II}$); 2.09–1.95 (m, 66H, C$H_3$CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–168.8 (CH_3*C*O); 101.8 (2C) (C-1$^{Glc\ II,\ Gal\ II}$); 100.7 (2C) (C-1$^{Glc\ III,\ IV}$), 99.7 (C-1$^{Glc\ I}$); 98.4, 97.5, 96.1 (C-1$^{Xyl\ II,\ III,\ IV}$); 76.4, 76.3, 75.6, 75.4, 75.0, 74.5, 73.9, 73.5, 72.6, 72.2 (2C); 72.1, 71.9 (2C); 71.5, 70.9 (2C); 70.5, 69.6, 69.5 (3C); 69.3 (3C); 68.5, 68.3, 67.6, 67.2, 65.8, 65.7, 64.1 (C-2$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-3$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-4$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-5$^{Glc\ I,\ II,\ III,\ IV,\ Gal\ II}$, C-6$^{Glc\ II,\ III,\ IV}$); 62.1, 61.6 (C-6$^{Glc\ I,\ Gal\ II}$); 59.3, 59.0 (2C) (C-5$^{Xyl\ II,\ III,\ IV}$); 21.2–20.57 (*C*$H_3$CO). HRMS (ESI) calcd for $C_{89}H_{120}O_{60}Na^+$: 2171.6237, found: 2171.6187.

*2.2.8. [(2,3,4-Tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4-tri-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-[(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→2)-(3,4-di-O-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-O-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-O-acetyl-α-D-glucopyranosyl fluoride (**12**) per(OAc)-XXLG-αF*

The title compound **12** was obtained from compound **11** (1.45 g, 674 μmol) as previously described for the preparation of compound **8**. Purification by flash chromatography on silica gel previously neutralized with triethylamine (1:2 toluene/ethyl acetate) afforded the title compound **12** (765 mg, 356 μmol, 53% yield). LRMS (MALDI): m/z = 2174 $[M+Na]^+$. $[\alpha]_D$ = +57 (c = 1.0, chloroform). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 5.62 (dd, $J_{1,2}$ = 2.7 Hz and $J_{C,F}$ = 53.1 Hz, 1H, H-1$^{Glc\ I}$); 5.44 (t, J = 9.8 Hz, 1H, H-3$^{Glc\ I}$); 5.42–5.27 (m, 4H, H-3$^{Glc\ IV,\ Xyl\ II,\ III,\ IV}$); 5.21–4.86 (m, 13 H, H-1$^{Xyl\ II,\ III,\ IV}$, H-2$^{Glc\ I,\ II,\ Gal\ II}$, H-3$^{Glc\ II,\ III}$, H-4$^{Glc\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$); 4.83–4.65 (m, 8H, H-1$^{Glc\ III,\ IV,\ Gal\ II}$, H-2$^{Glc\ III,\ IV,\ Xyl\ III,\ IV}$, H-3$^{Gal\ II}$); 4.61–4.56 (m, 2H, H-1$^{Glc\ II}$, H6a$^{Glc\ I}$); 4.15–4.12 (m, 2H, H-6$^{Gal\ II}$); 4.10–3.98 (m, 3H, H-5$^{Glc\ I}$, H-6a$^{Glc\ III}$, H-6b$^{Glc\ I}$); 3.96–3.74 (m, 13H, H-2$^{Xyl\ II}$, H-4$^{Glc\ I,\ II,\ III}$, H-5$^{Glc\ IV,\ Xyl\ III,\ Gal\ II}$, H-5a$^{Xyl\ II}$, H-6$^{Glc\ II}$, H-6a$^{Glc\ IV}$, H-6b$^{Glc\ III}$); 3.71–3.60 (m, 5H, H-5$^{Glc\ III,\ Xyl\ IV}$, H-5b$^{Xyl\ II}$, H-6b$^{Glc\ IV}$); 3.42 (m, 1H, H-5$^{Glc\ II}$); 2.17–1.94 (m, 66H, C$H_3$CO). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) = 170.7–168.7 (CH_3CO), 103.9 (d, $J_{C,F}$ = 227.6 Hz, C-1$^{Glc\ I}$); 101.5 (C-1$^{Gal\ II}$); 100.8, 100.6, 100.5 (C-1$^{Glc\ II,\ III,\ IV}$); 98.5 (C-1$^{Xyl\ II}$); 97.5 (C-1$^{Xyl\ III}$); 96.1 (C-1$^{Xyl\ IV}$); 75.9, 75.6, 75.2 (C-4$^{Glc\ I,\ II,\ III}$); 74.4 (2C); 74.0, 73.5, 73.2, 72.5, 72.1 (2C); 72.0, 71.9, 71.5, 71.2, 70.9, 70.7, 70.6, 70.5, 69.6, 69.5 (2C); 69.4 (2C); 69.3, 69.2 (2C); 68.4, 67.1 (C-2$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-3$^{Glc\ I,\ II,\ III,\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-4$^{Glc\ IV,\ Xyl\ II,\ III,\ IV,\ Gal\ II}$, C-5$^{Glc\ I,\ II,\ III,\ IV,\ Gal\ II}$); 67.6 (C-6$^{Glc\ IV}$); 66.7, 66.5 (C-6$^{Glc\ II,\ III}$); 61.4 (2C) (C-6$^{Glc\ I,\ Gal\ II}$); 59.3 (2C), 59.0 (C-5$^{Xyl\ II,\ III,\ IV}$); 21.2–20.5 (*C*$H_3$CO). HRMS (ESI) calcd for $C_{89}H_{119}O_{59}FNa^+$: 2173.6193, found: 2173.6218.

*2.2.9. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl fluoride (**13**)*
XXLG-αF

Compound **12** (603 mg, 280 μmol) was de-*O*-acetylated by treatment with methanolic sodium methoxide as described for the preparation of compound **9**. Lyophilization afforded the title compound **13** (343 mg, 280 μmol, 100% yield). LRMS (MALDI): m/z = 1249 $[M+Na]^+$. HRMS (ESI) calcd for $C_{45}H_{75}O_{37}FNa^+$: 1249.3869, found: 1249.3895.

*2.2.10. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranose (**14**)*
XXLG

Compound **10** (70 mg, 32 μmol) was de-*O*-acetylated by treatment with methanolic sodium methoxide as described for the preparation of compound **6**. Lyophilization afforded the title compound **14** (29 mg, 24 μmol, 75% yield). LRMS (MALDI): m/z = 1247 $[M+Na]^+$. HRMS (ESI) calcd for $C_{45}H_{76}O_{38}Na^+$: 1247.3912, found: 1247.3921.

*2.2.11. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-α,β-D-glucopyranose (**15**)*
XXLGXXLG

H. insolens Cel7B E197A glycosynthase (3 mg) was added to a solution of compound **14** (40 mg, 33 μmol) and fluoride 13 (40 mg, 33 μmol) in carbonate/bicarbonate buffer (0.1 M, pH 10, 1.5 mL). After stirring overnight at 37 °C, more glycosynthase (3 mg) was added and the reaction mixture was stirred 48 h at 37 °C. The mixture was desalted with mixed bed resin and filtered. The solution was diluted with a mixture acetonitrile/water (7:3, 3 mL) and evaporated to dryness with silica gel. Purification by flash chromatography on silica gel (7:3 then 65:35 then 6:4 acetonitrile/water) followed by filtration through 45 μm and lyophilization afforded the title compound **15** (32% yield). LRMS

(MALDI): m/z = 2454 $[M+Na]^+$. HRMS (ESI) calcd for $C_{90}H_{150}O_{75}Na^+$: 2453.7822, found: 2453.7822.

*2.2.12. [α-D-Xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[β-D-galactopyranosyl-(1→2)-α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-α,β-D-glucopyranose (**3**)*
XXLGXXLGXXLG

The title compound **3** was prepared together with compound **15**. Purification by flash chromatography on silica gel (7:3 then 65:35 then 6:4 acetonitrile/water) followed by filtration through 45 μm and lyophilization afforded the title compound **3** (16% yield). LRMS (MALDI): m/z = 3661 $[M+Na]^+$. HRMS (ESI) calcd for $C_{135}H_{221}O_{112}(Na^+)_3$: 1235.7181, found: 1235.7263.

*2.2.13. β-D-Galactopyranosyl-(1→4)-β-D-glucopyranosyl)-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-α,β-D-glucopyranose (**18**)*
LacXXXGGGGGXXXG

H. insolens Cel7B E197A glycosynthase (6 mg) was added to a solution of undecasaccharide **16** (Fauré et al., 2006) (21 mg, 15.1 μmol, 1.3 equiv.) and fluoride **17** (Fauré et al., 2006) (40 mg, 32.6 μmol, 1.0 equiv.) in carbonate/bicarbonate buffer (0.1 M, pH 10, 3.0 mL) and the solution was stirred overnight at 37 °C. The mixture was desalted with mixed bed resin and filtered. The filtrate was diluted with a mixture acetonitrile/water (7:3, 5 mL) and evaporated to dryness with silica gel. Purification by flash chromatography on silica gel (7:3 then 65:35 then 6:4 acetonitrile/water) followed by filtration through 45 μm and lyophilization afforded the title compound **18** (72% yield). LRMS (MALDI): m/z = 3102 $[M+Na]^+$. HRMS (ESI) calcd for $C_{114}H_{190}O_{95}Na^+$: 3101.7822, found: 3101.9858.

2.3. Binding assays

Adsorption experiments were conducted in deionized water containing 0.02% thimerosal (pH 5.5). Wet bacterial microcrystalline cellulose (BMCC) was prepared as previously described (Lopez et al., 2010). Typically 12% (dry weight) BMCC, was used as cellulosic material (approximately exactly 20 mg) and solutions of XGOs with different concentration (5–2000 μg mL^{-1}) were prepared by dilution of a 2 mg mL^{-1} XGO solution. Cellulose was mixed with 1.5 mL of each XGO solution using glass balls, until homogenous suspension was obtained to improve the repeatability. Mixtures were incubated for 15 h, optimised time resulting from kinetic studies (results not shown) at 40 °C under head-over-tail mixing. Samples were centrifuged (15 min, 20,000 × g) and the amount of unbound material in the supernatants was quantified by determination of the total sugar content using the automated colorimetric orcinol method (Tollier & Robin, 1979). The amount of adsorbed material was determined from the difference in the amount of XGs present in the supernatant before and after incubation. Blank, obtained by incubation of cellulose in water with 0.02% thimerosal without XG, was previously subtracted. Two to four measurements were made, depending on initial material availability. The average and the corresponding error measurements were calculated.

3. Results and discussion

3.1. Enzymatic synthesis of xyloglucooligosaccharides

It was already shown that structural variations on the side chains of XGs change the efficiency of binding to cellulose. For instance, XGs with reduced levels of galactosyl substitution present self-association that competes with the cellulose interactions while the modification of fucosyl residue numbers had little influence (Whitney et al., 2006) or strong contribution (Hayashi et al., 1994), depending of the authors. But, due to the modification of physical properties (solubility or conformational changes) of the polymer, the exact role of these glycosyl residues during the interaction cannot be evaluated.

Controlled enzymatic hydrolysis of tamarind xyloglucan by cellulases in an ultrafiltration cell allowed to isolate XGOs made of twelve glucosyl units in their backbones that may be substituted on their primary hydroxyls in a random fashion by α-D-Xyl*p*-(1→6) and β-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6) units (Lopez et al., 2010). These XGOs were found to bind quite efficiently to cellulose (Lopez et al., 2010).

To assess the influence of side chains on the interactions with BMCC, XXXG-XXXG-XXXG **1**, XLLG-XLLG-XLLG **2**, XXLG-XXLG-XXLG **3** and XXXG-GGGG-XXXG **4** were selected as target molecules (Fig. 1).

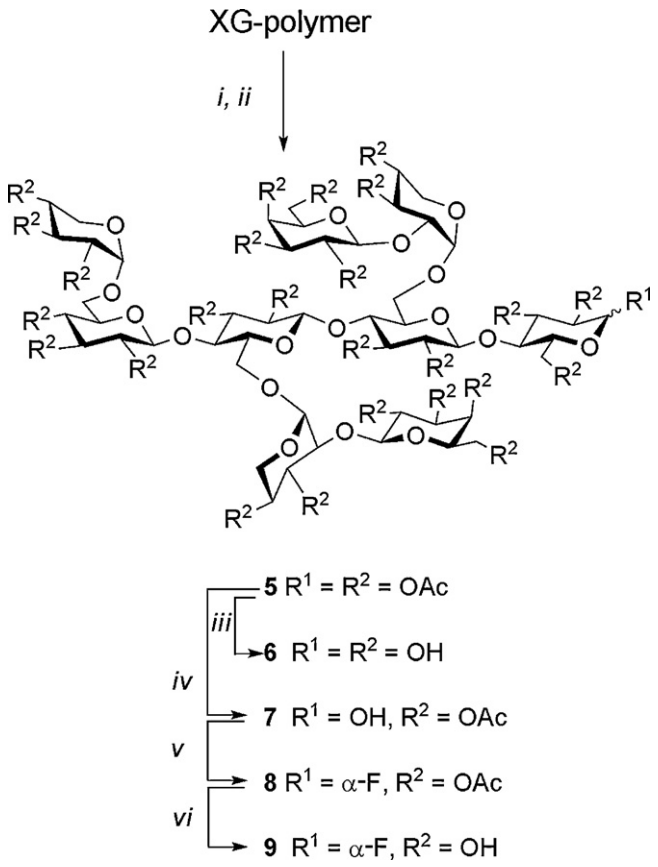
Compound **1** has been recently prepared using a new methodology based on full enzymatic degradation of tamarind XGs down to its repetitive motifs, enzymatic de-galactosylation to XXXG, chemical activation at the anomeric end with a fluorine atom and auto-condensation using the Cel7B E197A glycosynthase from *Humicola insolens* (Saura-Valls et al., 2006).

Glycosynthases are rather new type of enzymes generated by the mutation of catalytic carboxylate of retaining glycoside hydrolases by a non-nucleophile amino acid residue. These mutated enzymes are hydrolytically inactive enzymes, but they are still able to catalyze the transfer of sugar moieties from α-glycosyl fluorides (mimicking the glycosyl–enzyme intermediate for retaining β-glycoside hydrolases) onto acceptor molecules (Fort, Boyer, et al., 2000; Fort, Christiansen, et al., 2000; Mackenzie, Wang, Warren, & Withers, 1998; Malet & Planas, 1998).

In this work, we decided to develop the same strategy for the preparation of **2**–**4**.

Enzymatic degradation of XGs from tamarind seed with cellulase (Genencor 3042A) followed by acetylation gave protected XLLG **5** in 37% yield. XLLG **6** was obtained in 74% yield by catalytic de-*O*-acetylation of **5**. Standard hydrazine acetate treatment of **5** led to a selective acetyl removal at the anomeric position with a 59% yield. Compound **7** was treated with diethylaminosulfur trifluoride (DAST) to yield an anomeric mixture of fluorides which was anomerized with pyridine hydrofluoride into acetylated α-fluoride **8**. This key compound was isolated after flash chromatography in 81% yield over the two steps. Catalytic de-*O*-acetylation gave the potential donor **9** in 93% yield (Scheme 1).

However, Cel7B E197A cellosynthase failed to catalyze the enzymatic condensation of **9** to afford **2**. It may be speculated at this stage that galactosyl residues of compound **9** prevent any recognition of XLLG motif by the enzyme due to steric hindrance with the amino acids which constitute the −2 to +2 subsites.



Scheme 1. Synthesis of the key fluoride 9. (i) Cellulase, 37 °C, 24 h; (ii) Ac_2O/pyridine/DMAP, rt, 12 h; (iii) MeONa/MeOH, rt, 12 h; (iv) NH_2NH_2AcOH/DMF, 50 °C, 30 min; (v) DAST/CH_2Cl_2, −30 °C, 1 h, then HF/pyridine, −50 °C to −10 °C, 2.5 h.

It was known that kinetically controlled enzymatic treatment of XXLG, XLXG and XLLG by β-D-galactosidase from *A. niger* can be used to remove the galactosyl unit from the penultimate residue to produce a single galactosylated compound (York, Harvey, Guillen, Albersheim, & Darvill, 1993). By using this enzymatic procedure followed by acetylation of the reaction mixture and its purification, acetylated XXLG 10 was isolated in 45% yield.

This compound **10** was treated as already described for the preparation of 9. Selective *O*-deacetylation of the anomeric position gave **11** in 99% yield. DAST and HF/pyridine treatments afforded the acetylated α-fluoride **12** in 51% yield. Catalytic de-*O*-acetylation gave the expected donor **13**. Enzymatic auto-condensation of **13** in the presence of 1 equiv. of XXLG **14** (obtained by de-*O*-acetylation of **10**) gave the compound **3** and the hexadecasaccharide XXLGXXLG **15** with 16% yield and 25% yield, respectively (Scheme 2).

Since Cel7B E197A cellosynthase catalyzes the condensation of the fluoride 13, we can assume that the galactosyl residue at the penultimate unit of 9 was responsible for absence of recognition of this compound. To define whether the steric clash occurs at the −2 or +1 subsites, XLLG-αF **9** and XXLG-αF **13** were incubated with XXLG **14** and XLLG **6**, respectively. Only the coupling of XXLG-αF **13** and XLLG **14** gave condensation products (not shown), so it can be assessed that −2 subsite is responsible for the failure of the synthesis of 2.

To obtain the target molecule 4 we used the strategy of "blocked" donors, either with a tetrahydropyranyl (*O*-THP) group or with a lactosyl unit (Fauré et al., 2006; Fort, Christiansen, et al., 2000). The acceptor molecule GGGGXXXG **16** was already prepared (Fauré et al., 2006) (Scheme 3). The coupling of the "blocked" fluoride **17** (Fauré et al., 2006) with **16** afforded GalG-XXXG-GGGG-XXXG **18** in 71% yield. The hydrolysis of the lactosyl residue could, in theory, be done with the β-glucosidase from *Agrobacterium* sp. to afford the target compound **4** (Namchuk & Withers, 1995). However, due to the small amounts of material, it was assumed that the lactosyl residue would not have significant influence on interaction with cellulose thus **18** was directly used for the binding studies instead of the initially targeted molecule **4**.

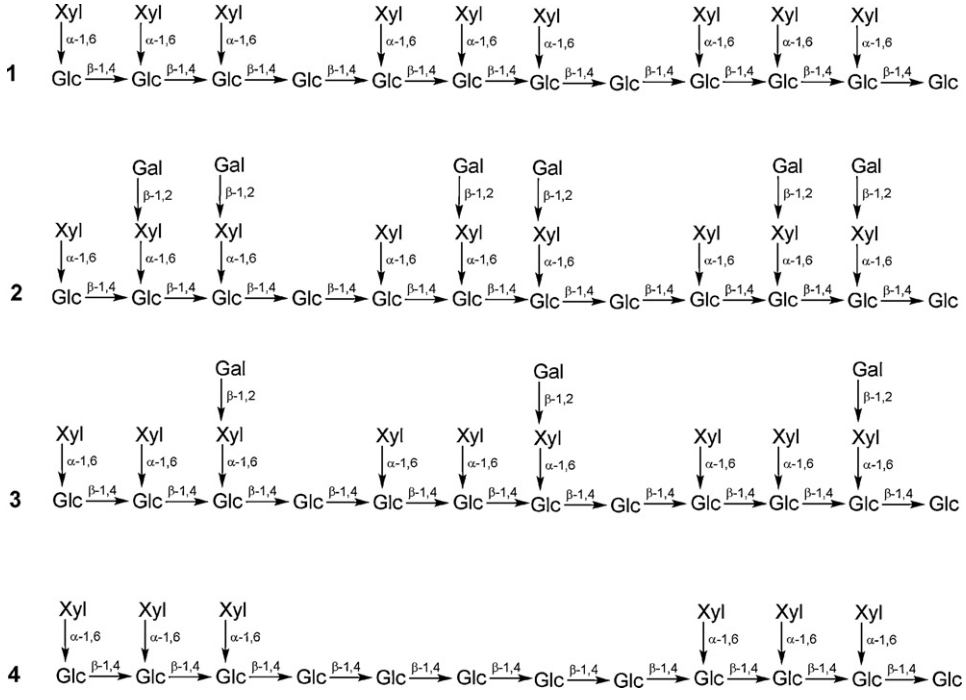
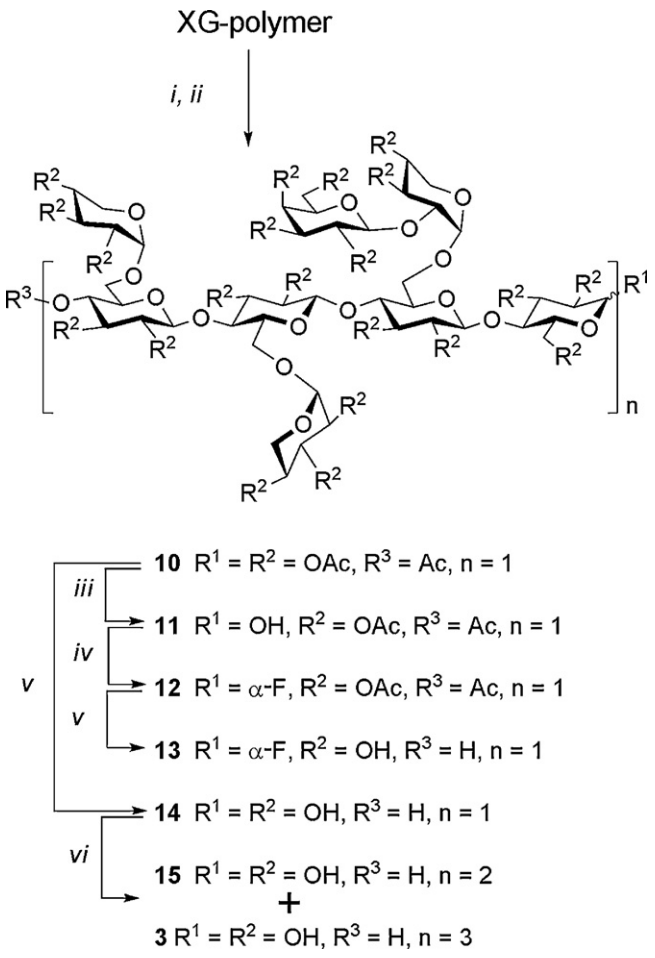


Fig. 1. Structures of the target molecules.



Scheme 2. Preparation of $(XXLG)_2$ 15 and $(XXLG)_3$ 3. (i) Cellulase, 37 °C, 24 h, then β-galactosidase, 25 °C, 2 × 48 h; (ii) Ac_2O/pyridine/DMAP, rt, 12 h; (iii) NH_2NH_2AcOH/DMF, 50 °C, 30 min; (iv) DAST/CH_2Cl_2, −30 °C, 2 h, then HF/pyridine, −50 °C to −10 °C, 2 h; (v) MeONa/MeOH, rt, 12 h; (vi) **13** + glycosynthase Cel7BE197A/carbonate buffer, 37 °C, 48 h.

Structure of the compounds was confirmed using ^1H and ^{13}C spectroscopy, by comparison with XGO models (Fauré et al., 2006; Fauré, Cavalier, Keegstra, Cottaz, & Driguez, 2007; Picard et al., 2000) and HRMS.

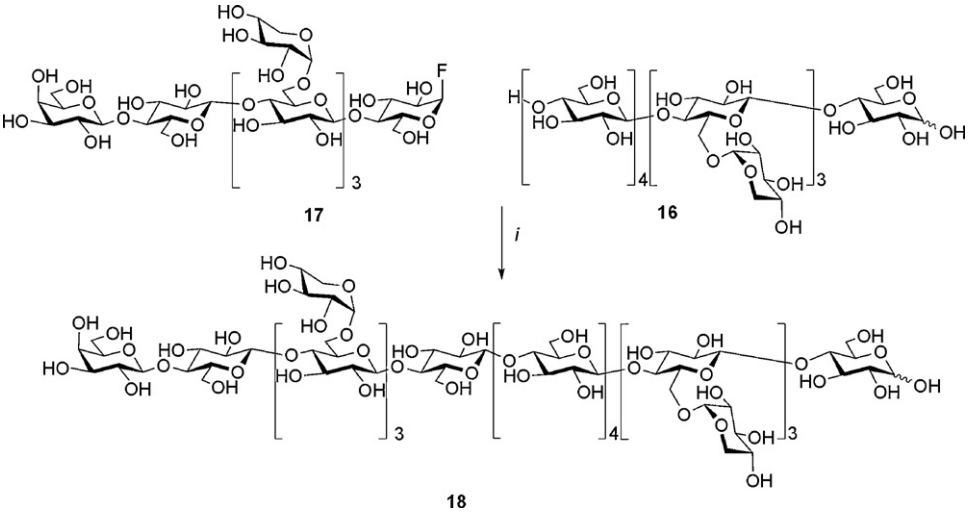
3.2. Interaction studies

The preparation of these different XGOs presenting a well defined structure allowed the study of their interactions with cellulose. These results gave some evidences concerning the influence of XG side chains on their interaction with cellulose. Interactions have been performed with BMCC as cellulosic material due to experimental constraints to determine adsorption isotherms and in order to compare with results already published (Lopez et al., 2010). Adsorption isotherms relative to chemo-enzymatically synthesized oligosaccharides are presented in Fig. 2.

General behavior of isotherms follows theoretical prediction of the Langmuir model similarly to the work previously done on controlled size XGOs (Lopez et al., 2010). A fast increase of adsorbed XGO quantity for low concentration of free material remaining in solution at equilibrium (C_e) is observed as a moderate increase characterized the adsorption for higher C_e. The equilibrium concentration where the change in adsorption is observed (around 170 μg mL^{-1}) is in accordance with the value observed previously for the mixture of $[XGO]_3$. $[XGO]_2$ curves are less steady than $[XGO]_3$ isotherms because of measure imprecision related by errors bars.

Firstly comparison of adsorption of $(XXXG)_2$ (Piens et al., 2007) and $(XXLG)_2$ on one hand and $(XXXG)_3$ **1** and $(XXLG)_3$ **3** on the other hand confirmed that XGOs constituted of twelve glucosyl residues on their backbone present the minimum length to observe significant interactions as previously described. Levels of adsorption determined for these well-defined structures are similar to those mentioned in this previous work.

Adsorption capacity of $(XXLG)_3$ **3** compared to $(XXXG)_3$ **1** is slightly higher. The difference of adsorption capacities of these two oligosaccharides is quite low. Although the phenomenon is minor, this evidences an influence of the antepenultimate galactosyl residue on the interaction capacity The same conclusion can be made regarding adsorption isotherms of $(XXXG)_2$ and $(XXLG)_2$. However since the adsorption level and consequently the accuracy are lower, the adsorption difference is not as significant as for the two other isotherms. These results give evidence of the minor influence of the galactosyl residue located on the antepenultimate glucosyl residue in XG–cellulose interactions. This is in agreement with previous work studying the *mur3* mutant phenotype (Madson et al., 2003) but are discordant with the conclusions published by Nguema-Ona et al. (2006), probably because of the low galactosylation level. This can suggest that galactosylation is significant from a certain substitution level.



Scheme 3. Preparation of Lac-XXXG-GGGG-XXXG 18. (i) Glycosynthase Cel7BE197A/carbonate buffer, 37 °C, 12 h.

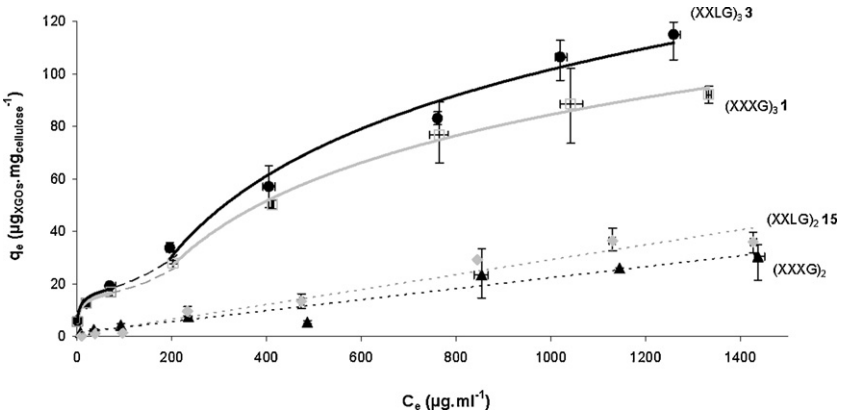


Fig. 2. Adsorption isotherms ($\mu g_{XGOs}\ mg_{cellulose}^{-1}$) of $(XXXG)_2$[28] (▲), $(XXLG)_2$ **15** (♦), $(XXXG)_3$ **1** (□) and $(XXLG)_3$ **3** (●) on BMCC.

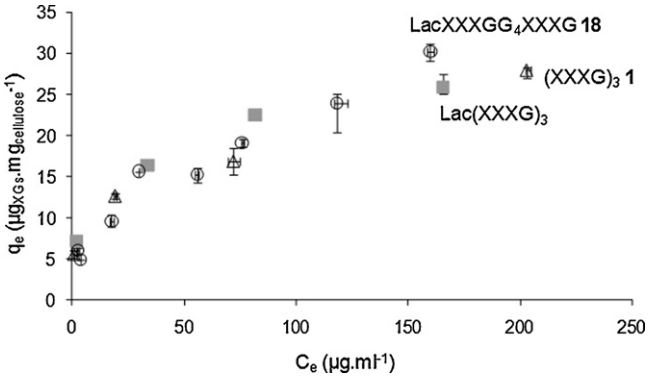


Fig. 3. Adsorption isotherms of $(XXXG)_3$ 1 (△), $Lac(XXXG)_3$ (■) and $LacXXXGG_4XXXG$ 18 (○) on BMCC.

To investigate the influence of a non substituted glucosyl residue on interactions, adsorption of $(XXXG)_3$ **1**, $Lac(XXXG)_3$ and $LacXXXGG_4XXXG$ **18** on BMCC was carried out. The $Lac(XXXG)_3$ was not *a priori* a target molecule however it was synthesized from oligosaccharide 1 as described in the literature (Fauré et al., 2006) to be able to determine the influence of the lactosyl residue. Adsorption isotherms obtained from these 3 oligosaccharides are represented in Fig. 3.

The isotherms obtained from compounds **1**, **18** and $Lac(XXXG)_3$ look like general isotherm and values for all the three experiments are extremely close.

Firstly, in order to investigate the potential role of lactosyl unit on interactions, isotherms of $(XXXG)_3$ and $Lac(XXXG)_3$ were considered. Similar adsorption capacity of both compounds was observed confirming the assumption that lactosyl residue has little influence on interactions. The influence of the linear fragment present in compound **18** was evaluated through the comparison of the isotherm obtained from compound **18** and the one from $Lac(XXXG)_3$ and $(XXXG)_3$. Compared to both fully xylosated XGOs, with and without lactosyl residue, the oligosaccharide **18**, containing a non substituted fragment, did not show any improvement in adsorption capacity. These results tend to prove that either the length of the non substituted fragment is too small or more probably that the presence of non substituted glucosyl residue did not improve the adsorption capacity.

4. Conclusion

XG–cellulose interaction studies reported so far in the literature could not clearly evidence the role of the density and distribution of XG side chains since the substrates with precise molecular structure are too difficult to isolate from biomass. This work reports the synthesis of well-defined XGOs by chemo-enzymatic approach relying on a cellosynthase-based polycondensation strategy. Through the synthesis, different tamarind XGOs were tested. Some of them, XXXG and XXLG, are recognized by glycosynthase *Hi*Cel7B E197A while XLLG and its α-fluorinated derivative are neither acceptor nor donor towards this enzyme. Digalactosylated compound are nevertheless recognized by other glycosynthases as already recently published (Piens et al., 2007). These results highlight the limited active site accessibility of *Hi*Cel7B E197A and give information of its topology. The chemo-enzymatic synthesis of a xylogluco-oligosaccharide, containing succession of non substituted glucosyl residues, was achieved.

Study of adsorption capacity of these chemo-enzymatically synthesized substrates on cellulose led to several conclusions on key factor governing XGs–cellulose interactions. First, as previously described (Lopez et al., 2010), twelve glucosyl residues are the minimum length necessary to observe interactions. We have given evidences of the minor variation of adsorption capacity in presence of the galactosyl residues on the penultimate residue. Finally, four consecutive non substituted glucosyl units did not modify amount of XGOs adsorbed on BMCC. These conclusions let appear the minor influence of the side chains on XG–cellulose interaction as determined by computational modelling (Hanus & Mazeau, 2006). This chemo-enzymatic synthesis could be applied to fucosylated XGOs, to investigate the role of trisaccharidic side chains.

Acknowledgments

Ms P. Chaud and S. Boullanger are thanked for technical assistance. This work was supported by CNRS and INRA especially through the GDR INRA/CNRS no. 2812 “Assemblages des Molécules Végétales”.

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