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Synthesis of a Library of Xylogluco-Oligosaccharides for Active-Site Mapping of Xyloglucan *endo*-Transglycosylase

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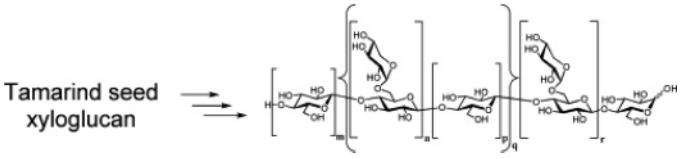

Complex oligosaccharides containing α-D-xylosyl-(1→6)-*β*-D-glucosyl residues and unsubstituted *β*-(1→4)-linked D-glucosyl units were readily synthesized using enzymatic coupling catalyzed by the Cel7B E197A glycosynthase from *Humicola insolens*. Constituting this library required four key steps: (1) preparing unprotected building blocks by chemical synthesis or enzymatic degradation of xyloglucan polymers; (2) generating the donor synthon in the enzymatic coupling by temporarily introducing a lactosyl motif on the 4-OH of the terminal glucosyl units of the xylogluco-oligosaccharides; (3) synthesizing the corresponding α-fluorides, followed by their de-*O*-acetylation and the glycosynthase-catalyzed condensation of these donors onto various acceptors; and (4) enzymatically releasing lactose or galactose from the reaction product, affording the target molecules in good overall yields. These complex oligosaccharides proved useful for mapping the active site of a key enzyme in plant cell wall biosynthesis and modification: the xyloglucan *endo*-transglycosylase (XET). We also report some preliminary enzymatic results regarding the efficiency of these compounds.

Introduction

Xyloglucan is a key structural polysaccharide in the cell walls of many plant species[1−4] and can also serve as a plant energy reserve.[5,6] It consists of a cellulose-like main chain of *β*-(1→4)-linked D-glucosyl residues that is regularly substituted at C-6 with α-D-xylosyl and *β*-D-galactosyl-(1→2)-α-D-xylosyl residues. In the cell wall, some of the 2-hydroxyl groups of the *β*-D-galactosyl units are substituted with α-L-fucosyl residues.[7] The biosynthesis and the degradation of this major hemicellulosic polysaccharide are important in the plant life cycle and for industrial applications.[8]

Xyloglucan is hydrolyzed *in vitro* and *in vivo* by microbial and plant *endo*-*β*-1,4-glucanases belonging to glycoside hydrolase (GH) families[9] 5, 7, 12, 16, and 74. Hydrolysis yields branched xylogluco-oligosaccharides (XGOs) in which the reducing unit is usually a nonsubstituted glucosyl residue.[10−13]

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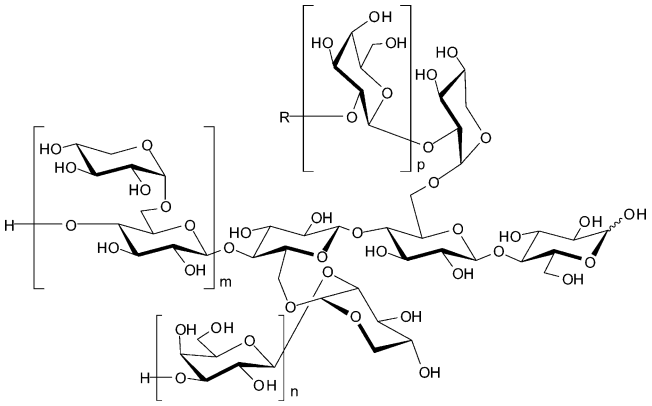

1 XXG m = n = p = 0, R = H
2 XXXG m = 1, n = p = 0, R = H
3 XXLG m = p = 1, n = 0, R = H
4 XLLG m = n = p = 1, R = H
5 XXFG m = p = 1, n = 0, R = α-L-Fuc

FIGURE 1. Structure of some common xyloglucan derivatives (XGOs) **1**−**5**.

Figure 1 gives the structures of some common XGOs. An unambiguous letter code is used for the nomenclature of each segment depending of the side chain: an unsubstituted D-Glc*p* is assigned G, α-D-Xyl*p*-(1→6)-*β*-D-Glc*p* segment is named X, and *β*-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-*β*-D-Glc*p* and α-L-Fuc*p*-(1→2)-*β*-D-Gal*p*-(1→2)-α-D-Xyl*p*-(1→6)-*β*-D-Glc*p* are respectively called L and F.[14]

In 1984, XGO **5**, but not **2** (Figure 1), was found to inhibit auxin-stimulated growth of etiolated pea stem segments.[15] A few years later, the total chemical syntheses of **2** and **5** and its precursors were described; however, poor overall yields precluded using chemical approaches to prepare larger oligosaccharides for structure/activity relationship studies.[16,17] Recent discoveries of a group of enzymes involved in cell wall modification have reopened the importance of accomplishing these synthesis.

Plants have evolved a unique set of GH family 16 enzymes to accomplish transient cell-wall modification in the absence of xyloglucan hydrolysis. These enzymes, the xyloglucan *endo*-transglycosylases (XETs, EC 2.4.1.207), can mediate molecular grafting reactions between xyloglucan chains[18−21] allowing reorganization of the network as cell morphology changes.[22] Plant genomic studies have identified numerous (30−40) sequence variants of XET-like genes and found that each gene is specifically regulated.[23−26] Thus, corresponding enzymes may have unique physiological roles based on substrate specificity or catalytic mechanism. A better understanding of the fundamental mechanisms contributing to the assembly and growth of the cell wall, in particular the cellulose−xyloglucan network, may forward engineering of novel polysaccharide composites with valuable physical properties by chemoenzymatic modification of cellulose surface.[13,27−29]

The XET mechanism, which occurs in two steps, involves attack on a glycosyl donor substrate to form a glycosyl-enzyme intermediate that is degraded by glycosyl transfer to a suitable acceptor substrate.[30] In vitro studies have demonstrated that XETs can use the xyloglucan polysaccharide as a donor in combination with xyloglucan or short xyloglucan oligosaccharides, such as XXG **1** and XLLG **4** (Figure 1), as acceptors.[31−33] Although xyloglucan is used as the donor in some XET assays,[26] its polydispersive nature (in terms of molecular mass and side-chain substitution) limits its use as a substrate in detailed enzyme kinetic analysis. The tetradecasaccharide XXXGXXXG **35** has been identified as a donor for the mixed-function XET/xyloglucanase (EC 2.4.1.207/EC 3.2.1.151) from nasturtium.[34] Encouraged by this discovery, we developed a new activity assay based on capillary electrophoresis to evaluate low molecular mass xylogluco-oligosaccharides as XET donors.[35] In addition, we started synthesizing a library of well-defined, low molecular mass donors for kinetic analysis and subsite mapping studies of XETs.

Results and Discussion

Glycosyl fluorides and nitrophenyl glycosides with the same anomeric configuration as the natural substrate are potent artificial donors in transglycosylation reactions catalyzed by retaining glycoside hydrolases and transglycosylases.[36,37] In a preliminary study, we synthesized the XXXG *β*-2-chloro-4-nitrophenyl and *β*-fluoride derivatives as potential donor

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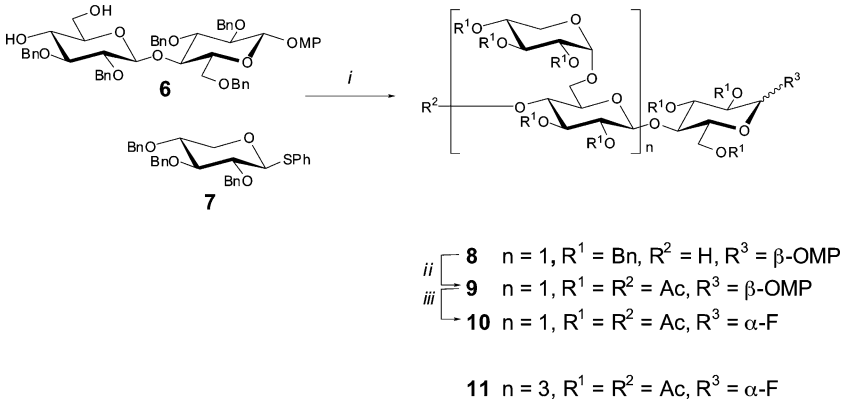
SCHEME 1[a]

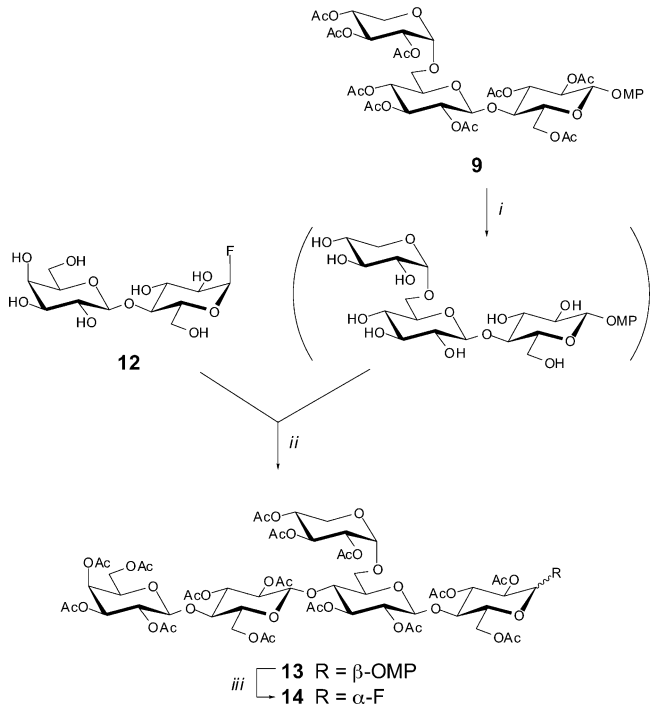


[a] Reagents and conditions: (i) (1) NIS, Et_2O/CH_2Cl_2, (2) TMSOTf; (ii) (1) H_2, $Pd(OH)_2$−C, MeOH/THF; (2) Ac_2O/pyridine, DMAP; (iii) (1) CAN, toluene/CH_3CN/H_2O, (2) DAST, CH_2Cl_2, (3) hydrogen fluoride/pyridine.

substrates for recombinant *Populus tremula x tremuloides* (hybrid aspen) XET16A (PttXET16A). We observed no transglycosylation with these derivatives as donors (unpublished data) but found that the tetradecasaccharide XXXGXXXG is a donor for PttXET16A.[35] Assuming that this latter experimental result indicates that substrate interactions with positive subsites of PttXET16A are required, we prepared a range of xylogluco-oligosaccharides that potentially spanned the catalytic residues by binding both positive and negative subsites of PttXET16A.[33] This library included combinations of the α-D-xylosyl-(1→6)-β-D-glucosyl residues and unsubstituted β-(1→4)-linked D-glucosyl units; these were coupled to either end of the XXXG motif for enzyme subsite mapping studies using kinetic analysis. The complexity and repetitive structure of XGOs prompted an enzymatic approach using glycosynthases, glycosidase mutants capable of performing oligosaccharide synthesis.[38,39] Previously, we demonstrated the efficiency of 6^{II}-substituted α-cellobiosyl fluorides as substrates for coupling reactions catalyzed by the Cel7B E197A glycosynthase from *Humicola insolens*.[40] Our goal was to extend the utility of this enzyme in the synthesis of XGO analogues. The required building blocks (α-fluoroglycoside donors and oligosaccharide acceptors) were prepared either by chemical synthesis or by enzymatic hydrolysis of xyloglucan.

We obtained glycoside acceptor **6**[41] as described in the literature in six steps from cellobiose and the glycoside donor **7** by benzylation of the known parent compound.[42] Stereocontrolled and regioselective glycosylation of **6** with the phenyl thioxyloside **7** gave the expected trisaccharide **8** in moderate yield (43%, Scheme 1). The structure of **8** was confirmed by its quasi-quantitative conversion into the acetylated **9** and complete assignment of its ^1H NMR spectrum. Oxidative deprotection of the anomeric position, followed by DAST treatment, afforded an expected anomeric mixture of fluorides.[43] Anomerization with HF/pyridine gave the pure α-fluoride **10** in 74% yield over the last three steps.

SCHEME 2[a]



[a] Reagents and conditions: (i) MeONa/MeOH; (ii) (1) Cel7B E197A glycosynthase, carbonate buffer, (2) Ac_2O/pyridine, DMAP; (iii) (1) CAN, toluene/CH_3CN/H_2O, (2) DAST, CH_2Cl_2, (3) hydrogen fluoride/pyridine.

Because the multistep synthesis of XGOs achieved a poor overall yield,[17] we used specific enzymatic hydrolysis to optimize the preparation of XXG **1** and XXXG **2** from tamarind xyloglucan.[35] According to the literature the corresponding peracetylated α-fluoride **11** was prepared in good overall yield from XXXG **2** (Scheme 1).[35]

An obvious approach to obtain well-defined XGOs was the polycondensation of α-fluorides, obtained by de-*O*-acetylation of **10** and **11**, catalyzed by the Cel7B E197A glycosynthase from *H. insolens*; however, as has been previously observed for cellobiosyl fluoride,[40] this reaction gave mostly insoluble polymers with little formation of dimers and trimers (data not shown). It also has been shown that 4-*O*-substituted glucosyl or galactosyl units on the nonreducing end of the fluoride donors prevent this uncontrolled polymerization.[40,43,44] Based on this information, we developed a new concept of enzymatic protec-

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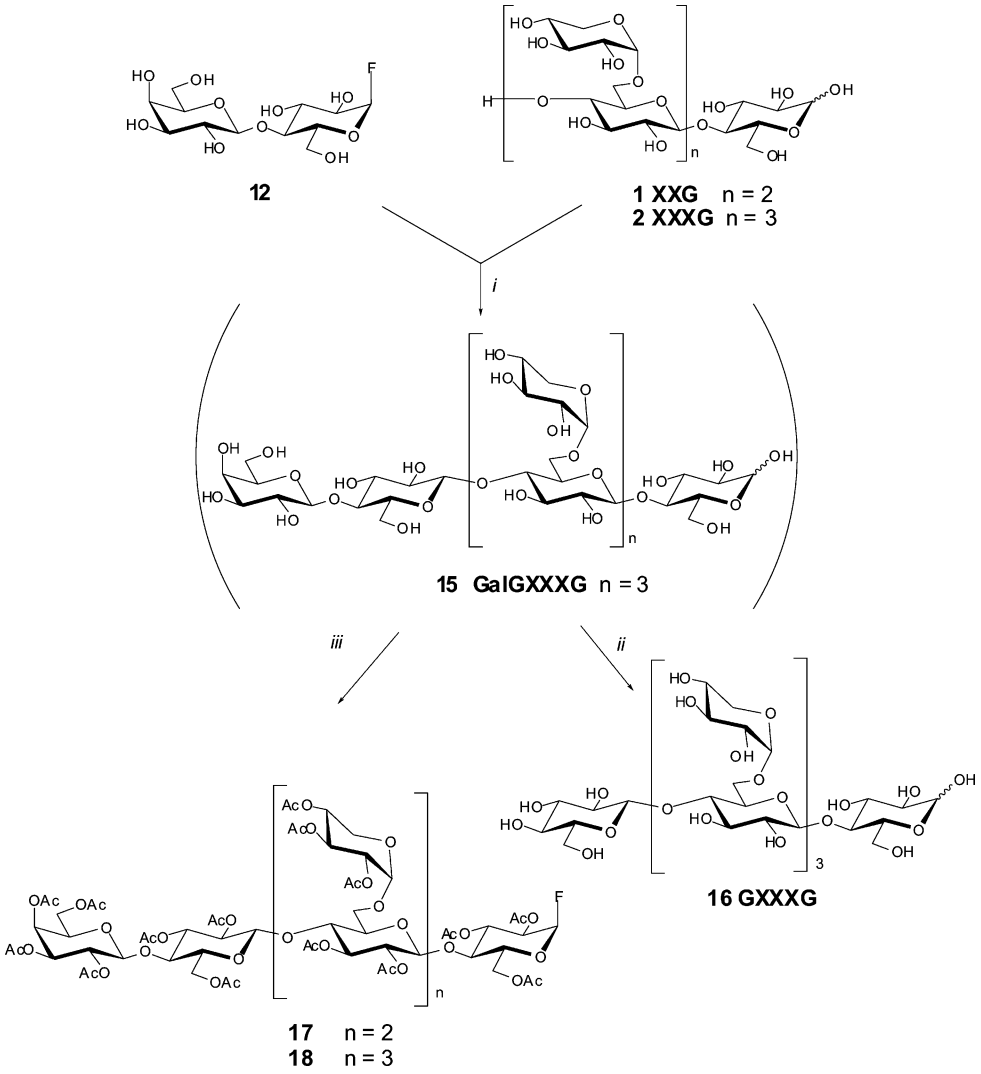
SCHEME 3[a]



[a] Reagents and conditions: (i) Cel7B E197A glycosynthase, carbonate buffer; (ii) *A. oryzae* β-galactosidase, acetate buffer; (iii) (1) Ac_2O/pyridine, DMAP, (2) hydrazine acetate, DMF, (3) DAST, CH_2Cl_2, (4) hydrogen fluoride/pyridine.

tion/deprotection of the nonreducing glucosyl unit of the (xylo)-gluco-oligosaccharide donors. This new approach involved introducing a lactosyl unit (or alternatively a tetrahydropyranyl (*O*-THP) group) on the 4^{ω}-OH position of fluorides. After coupling, the lactosyl unit can be wholly or partially removed by β-galactosidase and β-glucosidase activities, while the 4^{ω}-*O*-THP can be removed by acid-catalyzed hydrolysis.

With this approach, the enzymatic condensation of α-lactosyl fluoride **12**[45] in the presence of the compound arising from de-*O*-acetylation of **9** gave the expected pentasaccharide isolated in good yield after acetylation (89%). The pentasaccharide **13** was transformed into its corresponding α-fluoride **14** by oxidative removal of the aglycon followed by DAST and HF/pyridine treatments, as described above; the overall yield over the three steps was 46% (Scheme 2).

Enzymatic condensation of **12** on acceptor **2** gave GalGXXXG **15** in 68% yield. Kinetically controlled enzymatic de-galactosylation of **15** using the commercially available β-galactosidase from *Aspergillus oryzae* gave GXXXG **16** in 51% yield from **15** (Scheme 3). XGOs obtained by enzymatic condensation of **12** on acceptors **1** and **2** also gave corresponding fluorides **17** and **18** in 46% and 56% overall yields using the methodology already described for compound **11**.[35]

Glycosynthase-catalyzed coupling of 4^{II}-*O*-THP-cellobiosyl fluoride **19**[44] and the XXXG acceptor **2** gave the nonasaccharide GGXXXG **20** in 78% yield after acidic hydrolysis of the THP group (Scheme 4). GGXXXG **20** can also act as a glycosynthase substrate and be elongated by repeating the condensation with 4^{II}-*O*-THP-cellobiosyl fluoride and acid hydrolysis of the THP group; after these two steps, the undecasaccharide GGGGXXXG **21** was obtained in 86% yield. The coupling of 4^{II}-*O*-THP-cellobiosyl fluoride **19** with **16** likewise afforded GGGXXXG **22** in 76% yield after acidic deprotection.

De-*O*-acetylation of compound of **14** gave a pentasaccharide donor that was coupled with the XXXG acceptor **2**, removal of the lactosyl moiety by treatment with the mixed-function β-glucosidase/galactosidase from *Agrobacterium* sp.,[46] afforded

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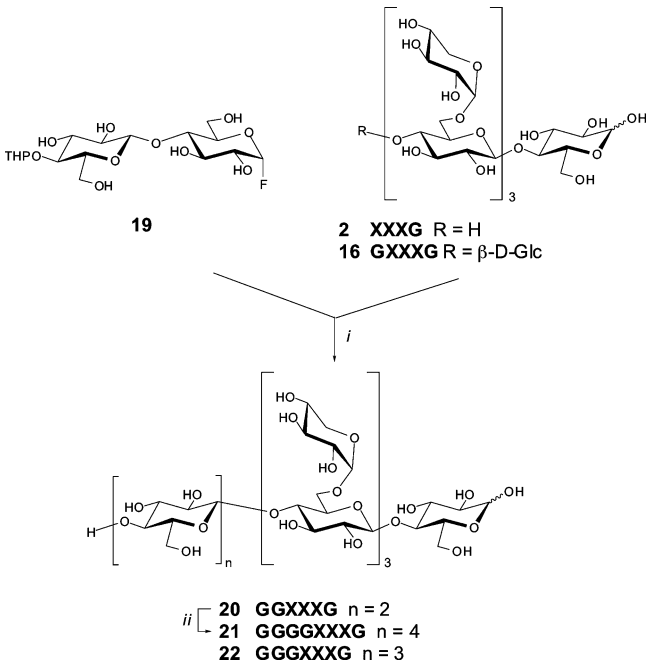
SCHEME 4[a]



[a] Reagents and conditions: (i) (1) Cel7B E197A glycosynthase, carbonate buffer; (2) 1 M HCl; (ii) (1) Cel7B E197A glycosynthase, carbonate buffer + **19**; (2) 1 M HCl.

the decasaccharide XGXXXG **23** in 66% yield over the two steps. When this sequence of reactions was repeated with decasaccharide **23** as an acceptor, the tridecasaccharide XGXGXXXG **24** was isolated in 87% overall yield. Condensation of the same pentasaccharide donor and GXXXG **16** followed by de-lactosylation, as already described for **23**, gave the expected compound XGGXXXG **25** in 80% yield (Figure 2).

Under the same conditions, coupling of the fluoride resulting from de-*O*-acetylation of **17** with XXXG **2** gave a tetradecasaccharide which was either de-lactosylated to give XXGXXXG **26** in 79% yield or de-galactosylated as described for the preparation of **16** to give GXXGXXXG **27** in 35% yield (Figure 3).

Using the same glycosynthase coupling strategy, de-*O*-acetylation of the nonasaccharidyl fluoride **18** gave a new donor which reacted with cellobiose GG **28**, cellotetraose GGGG **29**, the trisaccharide XG **30**,[47] and the pentasaccharide XXG **1**[35] to give the corresponding **31**−**34** in excellent yields (Figure 4).

Structure and purity of the compounds were confirmed using HR-ESMS, and $^{1}H/^{13}C$ NMR analysis or quantitative HPLC-ESMS on 4-aminobenzoic acid ethyl ester derivatives.[49] The regio- and stereochemistry of the new glycosidic linkages formed during the enzymatic condensations were confirmed by enzymatic hydrolysis using wild-type Cel7B from *H. insolens.*

The ability of compounds **15** and **20**−**27** and **31**−**34** to act as glycosyl donors in the transglycosylation reaction catalyzed by PttXET16A[48] was assessed by high-performance capillary electrophoresis (HPCE)[35] using the 8-aminonaphthalene-1,3,6-trisulfonate conjugate of XXXG (XXXG-ANTS) as the acceptor substrate.

Table 1 summarizes the preliminary results obtained under standardized assay conditions, where are given the relative reactivities of XGO donor substrates in PttXET16A-catalyzed transglycosylation reactions using XXXG-ANTS as the acceptor substrate. The new donors can be grouped into two classes relative to the reference tetradecasaccharide XXXGXXXG **35**, in which the glycosidic bond of the internal unsubstituted

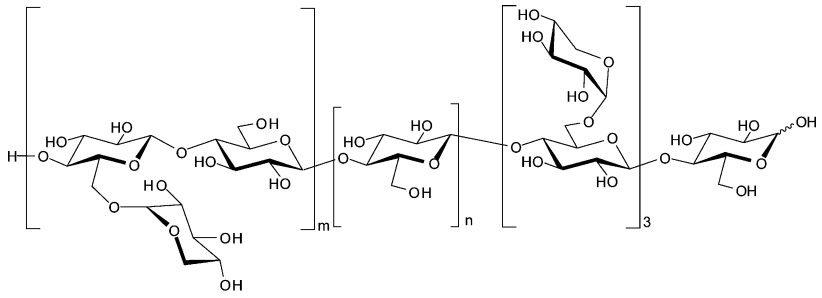


FIGURE 2. Structure of XGOs **23**−**25**.

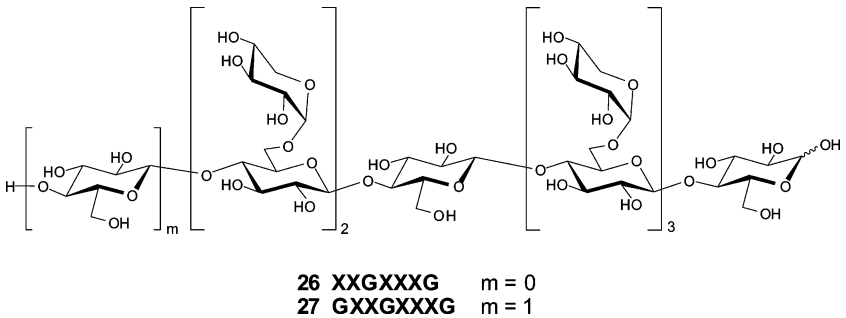
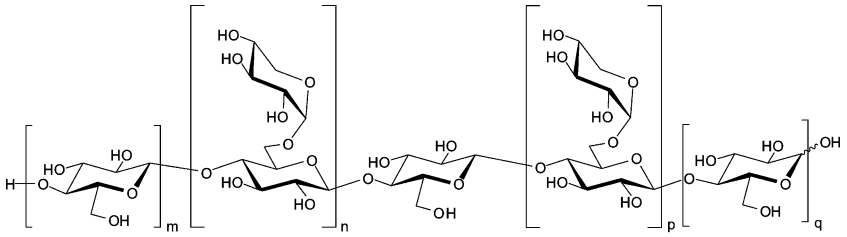


FIGURE 3. Structure of XGOs **26** and **27**.



28 GG m = 1, n = p = q = 0
29 GGGG m = 3, n = p = q = 0
30 XG m = 0, n = 1, p = q = 0
31 XXXGGG m = 0, n = 3, p = 0, q = 2
32 XXXGGGGG m = 0, n = 3, p = 0, q = 4
33 XXXGXG m = 0, n = 3, p = q = 1
34 XXXGXXG m = 0, n = 3, p = 2, q = 1
35 XXXGXXXG m = 0, n = 3, p = 3, q = 1

FIGURE 4. Structure of cello-oligomers **28** and **29** and XGOs **30**−**35**.

TABLE 1. Relative Reactivities of XGO Donor Substrates in PttXET16A-Catalyzed Transglycosylation Reactions Using XXXG-ANTS as the Acceptor Substrate

donor	initial rates[a] V_0/[E] (10^3 s^{-1})	reactivity relative to XXXGXXXG **35**[b] (%)
XXXGGG **31**	n.r.[c]	
XXXGXG **33**	n.r.	
XXXGXXG **34**	28.8	37
XXXGGGGG **32**	n.r.	
XXXGXXXG **35**	78.4	100
GXXGXXXG **27**	95.0	121
XGXGXXXG **24**	40.0	51
GGGGXXXG **21**	40.8	52
GGGXXXG **22**	20.4	26
XGGXXXG **25**	31.5	40
XXGXXXG **26**	20.9	27
XGXXXG **23**	1.0	1.3
GGXXXG **20**	0.5	0.6
GalGXXXG **15**	0.4	0.5

[a] Initial rates V_0/[E] were calculated from the peak areas of products in the electropherograms when monitoring the reaction for 15−30 min, using Man-ANTS (ANTS-derivatized mannose) as an internal standard.[35] [b] Relative reactivity: initial rate of transglycosylation of various donors relative to the rate of donor XXXGXXXG (**35**). [c] n.r., no reaction detected.

glucosyl (G) residue is cleaved to yield the transfer product XXXGXXXG-ANTS.[35] Those designed to probe the contribution of the positive enzyme subsites to XET catalysis have the general structure XXXG-nnnn; those designed to target negative subsites have the general structure nnnn-XXXG. We identified compounds GalGXXXG **15** and GGXXXG **20** as the smallest XET donors described so far, providing new insight into the substrate specificity of PttXET16A. More detailed comparative kinetic analysis for this new series of compounds will allow mapping of both donor and acceptor subsites of the binding cleft of the enzyme.

Conclusion

A combination of enzymatic and chemical steps, exploiting the versatility of wild-type glycoside hydrolases together with the *H. insolens* Cel7B E197A glycosynthase-assisted synthesis has proven to be a powerful approach for the preparation of a library of xyloglucan oligosaccharides from tamarin seed xyloglucan. A block synthesis strategy has been achieved by employing lactosyl or 4^{II}-*O*-THP-cellobiosyl building blocks as temporary protecting group, allowing the oligomerization of xyloglucan substructures catalyzed by the Cel7B E197A glycosynthase. The variety and the complexity of these well-defined xyloglucan oligomers constitute and unprecedented library of oligosaccharides useful for the biochemical characterization of xyloglucan *endo*-transglycosylases (XET), an important class of enzymes involved in the cell wall polysaccharide biosynthesis and modification in plants, and of practical importance in the chemoenzymatic surface modification of cellulose for the production of new composite biomaterials.

Experimental Section

Phenyl 2,3,4-Tri-*O*-benzyl-1-thio-β-D-xylopyranoside (7). To a solution of phenyl 1-thio-β-D-xylopyranoside[42] (0.97 g, 4.0 mmol) in DMF (30 mL) were added NaH (0.55 g, 24 mmol, 6 equiv) and a catalytic amount of Bu_4NI (10 mg). The mixture was vigorously stirred for 15 min at 0 °C, and then BnBr (4.3 mL, 36 mmol, 9 equiv) was added dropwise. After the solution was stirred for 30 min at 0 °C and 12 h at rt, MeOH and then Et_3N (2 mL) were added, and the resulting mixture was evaporated. The residue was dissolved in CH_2Cl_2, washed with water, dried, and concentrated under reduce pressure. Flash chromatography (cyclohexane/Et_2O, 9:1 v/v) of the residue gave the expected phenyl thioxyloside **7** (1.71 g, 3.3 mmol) in 83% yield, which crystallized in EtOH: mp 51−53 °C; $[\alpha]^{25}_D = +7$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.57−7.28 (m, 20H, S*Ph* and CH_2*Ph*), 4.94, 4.90, 4.87, 4.79, 4.74 (5d, $^2J_{H,H} = 11.0, 10.5, 11.0, 10.5, 11.7$ Hz, 5H, 5 C*H*$_2$Ph), 4.72 (d, $J_{1,2} = 9.4$ Hz, 1H, H-1), 4.65 (d, $^2J_{H,H} = 11.7$ Hz, 1H, C*H*$_2$Ph), 4.10 (dd, $J_{4,5a} = 4.6$ and $J_{5a,5b} = 11.5$ Hz, 1H, H-5a), 3.72−3.63 (m, 2H, H-3 and H-4), 3.49 (t, $J_{2,3} = 8.6$ Hz, 1H, H-2), 3.29 (dd, $J_{4,5b} = 9.7$ Hz, 1H, H-5b); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 131.8−127.5 (S*Ph* and CH_2*Ph*), 88.3 (C-1), 85.2 (C-3 or C-4), 80.4 (C-2), 77.7 (C-3 or C-4), 75.5, 75.3, 73.1 (*C*H$_2$Ph), 67.4 (C-5); DCI-MS *m*/*z* 530 $[M + NH_4]^+$. Anal. Calcd for $C_{32}H_{32}O_4S$: C, 74.97; H, 6.29; S, 6.25. Found: C, 75.00; H, 6.38; S, 6.55.

4-Methoxyphenyl [(2,3,4-Tri-*O*-benzyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). A solution of phenyl thioxyloside **7** (616 mg, 1.20 mmol, 1.2 equiv) and diol acceptor **6** (899 mg, 1.00 mmol) in Et_2O/CH_2Cl_2 (7:3 v/v, 65 mL) was stirred 15 min at

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0 °C under argon, sheltered from light, in the presence of *N*-iodosuccinimide (345 mg, 1.50 mmol, 1.5 equiv). Then a solution of TMSOTf (93 μL, 0.50 mmol, 0.5 equiv) in CH_2Cl_2 was added. After being stirred for 3 h at 0 °C, under the same conditions, the mixture was diluted with CH_2Cl_2, filtered through diatomaceous earth, washed with saturated aqueous sodium hydrogen carbonate then 10% (m/v) aqueous sodium thiosulfate, dried, and evaporated. Flash chromatography (cyclohexane/EtOAc, 3:1 v/v), followed by column chromatography using silica gel Si 60 (63−200 μm; toluene/EtOAc, 9:1 v/v), gave the trisaccharide **8** (555 mg, 0.43 mmol) in 43% yield: $[\alpha]^{25}_D = +52$ ($c = 1.2$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−7.20 (m, 40H, CH_2Ph), 7.02−6.77 (m, 4H, C_6H_4-OCH_3), 4.97−4.37 (m, 16H, CH_2Ph), 4.83 (d, $J_{1,2} = 7.7$ Hz, 1H, H-1 of Glc^I), 4.52 (d, $J_{1,2} = 3.8$ Hz, 1H, H-1 of Xyl^{II}), 4.49 (d, $J_{1,2} = 7.8$ Hz, 1H, H-1 of Glc^{II}), 4.00−3.09 (m, 17H, H-2 to H-6 of Glc and H-2 to H-5 of Xyl), 3.76 (s, 3H, $C_6H_4OCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.3, 151.6 (C-1 and C-4 of $C_6H_4OCH_3$), 139.1−138.5 (C-1 of CH_2Ph), 128.5−127.3 (others C aromatic of CH_2Ph), 118.5, 114.5 (others C aromatic of $C_6H_4OCH_3$), 102.8 (C-1 of Glc^I), 102.4 (C-1 of Glc^{II}), 98.3 (C-1 of Xyl^{II}), 84.4, 82.7, 81.8, 81.5, 81.4, 79.4, 77.8, 76.8, 75.8, 75.1, 72.0 (C-2 to C-5 of Glc and C-2 to C-4 of Xyl), 75.7, 75.4, 75.2, 75.0 (broad), 73.8, 73.3, 73.2 (CH_2Ph), 70.9, 68.1, 60.2 (C-5 of Xyl^{II} and C-6 of Glc^{I,II}), 55.6 ($C_6H_4OCH_3$); DCI-MS *m*/*z* 1318 $[M + NH_4]^+$; ES-HRMS *m*/*z* calcd for $C_{80}H_{84}O_{16}Na$ $[M + Na]^+$ 1323.5657, found 1323.5647.

4-Methoxyphenyl (2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (9). To a solution of trisaccharide **8** (699 mg, 0.54 mmol) in MeOH/THF (1:1 v/v, 25 mL) was added a catalytic amount of $Pd(OH)_2$-C. After vigorous stirring for 12 h under H_2 (1 atm) at rt, the reaction mixture was filtered through diatomaceous earth and the solution concentrated under reduced pressure and acetylated (acetic anhydride/pyridine, 1:2 v/v, 15 mL) in the presence of a catalytic amount of (dimethylamino)pyridine (DMAP). After 12 h of stirring at rt, the reaction was quenched by addition of MeOH at 0 °C and concentrated under reduced pressure. Flash chromatography (EtOAc/petroleum ether, 2:3 v/v) of the residue gave the acetylated **9** (497 mg, 0.52 mmol) in 97% yield: $[\alpha]^{25}_D = +32$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) see Table A in the Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3−169.1 ($COCH_3$), 155.8, 150.9 (C-1 and C-4 of C_6H_4-OCH_3), 118.7, 114.5 (others C aromatic of $C_6H_4OCH_3$), 100.0, 100.0 (C-1 of Glc^{I,II}), 96.1 (C-1 of Xyl^{II}), 75.4 (C-4 of Glc^I), 73.0, 72.8, 72.7, 72.7, 71.7, 71.4, 70.9, 69.4, 69.0, 68.9 (C-2 to C-5 of Glc and C-2 to C-4 of Xyl), 67.2 (C-6 of Glc^{II}), 61.8 (C-6 of Glc^I), 59.0 (C-5 of Xyl^{II}), 55.7 ($C_6H_4OCH_3$), 20.8−20.5 ($COCH_3$); DCI-MS *m*/*z* = 976 $[M + NH_4]^+$; ES-HRMS *m*/*z* calcd for $C_{42}H_{54}O_{25}$-Na $[M + Na]^+$ 981.2852, found 981.2860.

(2,3,4-Tri-*O*-acetyl-α-D-xylopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Fluoride (10). A mixture of acetylated glycoside **9** (410 mg, 430 μmol) and ammonium cerium nitrate (CAN, 2.34 g, 4.3 mmol, 10 equiv) in toluene/acetonitrile/water (1:1.4:1 v/v/v, 34 mL) was stirred at rt for 1.5 h. The reaction mixture was diluted with CH_2Cl_2 and then successively washed with brine, saturated aqueous sodium hydrogen carbonate, and water, dried, concentrated, and purified by flash chromatography (EtOAc/petroleum ether, 3:2 v/v) to give the expected hydroxy compound (290 mg, 340 μmol) in 79% yield characterized only by mass spectroscopy: CI-MS *m*/*z* = 870 $[M + NH_4]^+$; ES-HRMS *m*/*z* calcd for $C_{35}H_{48}O_{24}Na$ $[M + Na]^+$ 875.2433, found 875.2429.

Diethylaminosulfur trifluoride (DAST, 124 μL, 940 μmol, 5 equiv) was added dropwise to a stirred solution of the hydroxy compound (160 mg, 188 μmol) in CH_2Cl_2 (8 mL) at −30 °C. The solution was stirred for 2 h, and then the temperature of the cooling bath was raised to rt and the solution was concentrated under reduced pressure. Flash chromatography (EtOAc/petroleum ether, 1:2 then 1:1 v/v) using silica gel neutralized with triethylamine of the residue gave corresponding fluoride as a mixture of α/β anomers (152 mg, 176 μmol) in 95% yield, characterized only by CI-MS (*m*/*z* = 872 $[M + NH_4]^+$).

In a plastic vessel, a solution of the α/β-fluorides (101 mg, 120 μmol) in hydrogen fluoride/pyridine (7:3 v/v, 1 mL) was stirred at −50 °C for 15 min, and then the temperature of the cooling bath was raised to −10 °C for 2.5 h. The solution was diluted with dichloromethane, and then poured into a plastic beaker containing an ice-cooled solution of ammonia in water (3 M). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (three times), dried, and concentrated under reduced pressure. Flash chromatography (cyclohexane/EtOAc, 2:1, 3:2 then 1:1 v/v) using silica gel neutralized with triethylamine of the residue gave the expected fluoride **10** (99 mg, 116 μmol) in 98% yield: $[\alpha]^{25}_D = +67$ ($c = 1.8$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) see Table B in the Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2−169.0 ($COCH_3$), 103.6 (d, $J_{C,F} = 229.7$ Hz, C-1 of Glc^I), 100.1 (C-1 of Glc^{II}), 96.1 (C-1 of Xyl^{II}), 74.6 (C-4 of Glc^I), 72.9, 72.8, 71.7, 70.8, 70.7 (d, $J_{C,F} = 4.1$ Hz), 70.3 (d, $J_{C,F} = 24.6$ Hz), 69.2 (broad), 69.1, 69.0 (C-2 to C-5 of Glc and C-2 to C-4 of Xyl), 67.6 (C-6 of Glc^{II}), 61.1 (C-6 of Glc^I), 58.9 (C-5 of Xyl^{II}), 20.9−20.5 ($COCH_3$); FAB-MS *m*/*z* = 893 $[M + K]^+$; ES-HRMS *m*/*z* calcd for $C_{35}H_{47}O_{23}FNa$ $[M + Na]^+$ 877.2390, found 877.2383.

4-Methoxyphenyl (2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-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)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (13). Compound **9** (192 mg, 0.2 mmol) was treated in methanol (30 mL) with sodium methoxide (1 M in methanol, 100 μL) for 5 h at 0 °C. The solution was neutralized with Amberlite IRN 120 (H^+), filtered, and then concentrated under reduced pressure. The residue dissolved in deionized water was freeze-dried to give the expected compound in quantitative yield (116 mg). This compound was only characterized by mass spectroscopy: FAB-MS *m*/*z* = 581 $[M + H]^+$; ES-HRMS *m*/*z* calcd for $C_{24}H_{36}O_{16}Na$ $[M + Na]^+$ 603.1901, found 603.1914.

To this trisaccharide (103 mg, 0.18 mmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 5 mL) were added *H. insolens* Cel7B E197A glycosynthase (2 mg) and lactosyl fluoride **12** (80 mg, 0.23 mmol, 1.3 equiv). The solution was placed in a rotative shaker for 20 h at 37 °C and then freeze-dried. The residue was acetylated (acetic anhydride/pyridine, 1:2 v/v, 24 mL) in the presence of a catalytic amount of DMAP. After 12 h of stirring at rt, the reaction was quenched by addition of MeOH at 0 °C, concentrated under reduced pressure, and coevaporated with toluene. Flash chromatography (toluene/acetone, 4:1 v/v) of the residue gave the pentasaccharide **13** (237 mg, 0.15 mmol) in 87% yield: $[\alpha]^{25}_D = +5$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) see Table C in the Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4−168.8 ($COCH_3$), 155.8, 150.8 (C-1 and C-4 of $C_6H_4OCH_3$), 118.7, 114.5 (others C aromatic of $C_6H_4OCH_3$), 101.3 (C-1 of Gal), 100.5 (C-1 of Glc^{II}), 100.3 (C-1 of Glc^{III}), 99.7 (C-1 of Glc^I), 97.1 (C-1 of Xyl^{II}), 76.8, 75.8, 74.7, 74.3, 73.4, 73.1, 72.5, 72.5, 72.1, 72.0, 71.9, 71.6, 71.1, 70.6, 70.5, 69.0, 68.9, 68.6, 66.6 (C-2 to C-5 of Glc and Gal, and C-2 to C-4 of Xyl), 64.8 (C-6 of Glc^{II}), 62.1, 62.0 (C-6 of Glc^{I,III}), 60.8 (C-6 of Gal), 59.0 (C-5 of Xyl^{II}), 55.6 ($C_6H_4OCH_3$), 21.0−20.4 (CO CH_3); MALDI-TOF-MS *m*/*z* = 1557 $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{66}H_{86}O_{41}Na$ $[M + Na]^+$ 1557.4542, found 1557.4541.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-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)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Fluoride (14). A mixture of acetylated glycoside **13** (223 mg, 145 μmol) and CAN (797 mg, 1.450 mmol, 10 equiv) in toluene/acetonitrile/water (1:1.4:1 v/v/v, 15 mL) was stirred at rt for 2 h. The reaction mixture was diluted with CH_2Cl_2 and then successively washed with brine, saturated aqueous sodium hydrogen carbonate, and water, dried, concentrated, and purified by flash chromatography (toluene/acetone, 3:1 v/v) to give the expected C1-hydroxy compound (161

mg, 113 μmol; MALDI-TOF-MS m/z = 1451 $[M + Na]^+$) in 78% yield. To a solution of this C-1 hydroxy compound (160 mg, 112 μmol) in CH_2Cl_2 (5 mL) at −30 °C was added DAST (75 μL, 550 μmol, 5 equiv) dropwise. After the mixture was stirred for 2 h, the temperature of the cooling bath was raised to rt, and then the reaction was quenched by addition of MeOH and the solution concentrated under reduced pressure. Flash chromatography (toluene/acetone, 4:1 v/v) using silica gel neutralized with triethylamine of the residue gave the expected mixture of α/β fluorides (103 mg, 72 μmol; MALDI-TOF-MS m/z = 1453 $[M + Na]^+$) in 64% yield. In a plastic vessel, a solution of these α/β-fluorides (60 mg, 42 μmol) in hydrogen fluoride/pyridine (7:3 v/v, 0.75 mL) was stirred at −50 °C for 15 min, and then the temperature of the cooling bath was raised to −10 °C for 2.5 h. The solution was diluted with dichloromethane and then poured into a plastic beaker containing an ice-cooled solution of ammoniac (3 M). The organic layer was washed with saturated aqueous sodium hydrogencarbonate (three times), dried, and concentrated under reduced pressure. Flash chromatography (toluene/acetone, 3.5:1 v/v) using silica gel neutralized with triethylamine of the residue gave the expected fluoride **14** (56 mg, 39 μmol) in 93% yield: $[\alpha]^{25}_D$ = +30 (c = 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) see Table D in the Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4−168.8 (*C*OCH$_3$), 103.6 (d, $J_{C,F}$ = 229.8 Hz, C-1 of Glc^{I}), 101.3 (C-1 of Gal), 100.4 (C-1 of Glc^{II}), 100.2 (C-1 of Glc^{III}), 97.1 (C-1 of Xyl), 75.9, 75.7, 74.7, 74.3, 73.1, 72.5, 72.2, 72.0, 71.6, 71.1, 70.7, 70.5, 70.4, 70.4, 69.4, 69.0, 68.9, 68.6, 66.6 (C-2 to C-5 of Glc and Gal, and C-2 to C-4 of Xyl), 65.1 (C-6 of Glc^{II}), 62.1 (C-6 of Glc^{III}), 61.2 (C-6 of Glc^{I}), 60.9 (C-6 of Gal), 59.1 (C-5 of Xyl^{II}), 20.9−20.4 (CO*C*H$_3$); MALDI-TOF-MS m/z = 1453 $[M + Na]^+$; ES-HRMS m/z calcd for $C_{59}H_{79}O_{39}FNa$ $[M + Na]^+$ 1453.4080, found 1453.4081.

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)-D-glucopyranose (GalGXXXG 15). *H. insolens* Cel7B E197A glycosynthase (5 mg) was added to a solution of lactosyl fluoride **12** (0.31 g, 0.89 mmol, 1.2 equiv) and heptasaccharide **2** (0.79 g, 0.74 mmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 15 mL). The solution was placed in a rotative shaker for 20 h at 37 °C and then freeze-dried. The reaction mixture was diluted in acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute **15** (0.70 g) in 68% yield: MALDI-TOF-MS m/z = 1409 $[M + Na]^+$; ES-HRMS m/z calcd for $C_{51}H_{86}O_{43}Na$ $[M + Na]^+$ 1409.4441, found 1409.4439; LC/ES-MS analysis gave m/z 1558 $[M(ABEE) + Na]^+$; t_R = 16.36 min.

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)-D-glucopyranose (GXXXG 16). A solution of nonasaccharide **15** (56.0 mg, 40.4 μmol) in acetate buffer (0.1 m, pH 4.6, 2 mL) was treated with commercially available β-galactosidase (*A. oryzae*, 5 mg, 40 units) for 2.5 h at 30 °C. The reaction mixture was diluted in acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the octasaccharide **16** (25.0 mg, 20.4 μmol) in 51% yield: ^{1}H NMR (D_2O, 400 MHz) δ = 5.22 (d, $J_{1,2}$ = 3.7 Hz, 0.4H, H-1 of Glc$^{I\alpha}$), 4.95 (d, $J_{1,2}$ = 3.7 Hz, 3H, H-1 of Xyl), 4.66 (d, $J_{1,2}$ = 7.9 Hz, 0.6H, H-1 of Glc$^{I\beta}$), 4.58−4.54 (m, 3H, H-1 of Glc^{II,III,IV}), 4.51 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1 of Glc^{V}); MALDI-TOF-MS m/z = 1247 $[M + Na]^+$; ES-HRMS m/z calcd for $C_{45}H_{76}O_{38}Na$ $[M + Na]^+$ 1247.3912, found 1247.3910.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-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-tri-*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 (17). *H. insolens* Cel7B E197A glycosynthase (3 mg) was added to a solution of lactosyl fluoride **12** (151 mg, 0.44 mmol, 1.3 equiv) and pentasaccharide **1** (259 mg, 0.34 mmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 6 mL). The solution was placed in a rotative shaker for 20 h at 37 °C and then freeze-dried. The residue was acetylated (acetic anhydride/pyridine, 1:2 v/v, 24 mL) in the presence of a catalytic amount of DMAP. After 12 h of stirring at rt, the reaction was quenched by addition of MeOH at 0 °C, and the resulting solution was concentrated under reduced pressure and coevaporated with toluene. Flash chromatography (toluene/acetone, 3.5:1 v/v) of the residue gave the expected acetylated heptasaccharide (500 mg, 0.26 mmol) in 75% yield characterized by MALDI-TOF-MS: m/z = 1997 $[M + Na]^+$.

A mixture of this compound (479 mg, 243 μmol) and hydrazine acetate (34 mg, 369 μmol, 1.5 equiv) in DMF (5 mL) was stirred at 50 °C for 25 min. The solution was diluted with ethyl acetate, washed with brine (two times), dried, concentrated, and coevaporated with toluene. Flash chromatography (toluene/acetone, 3:1 v/v) of the residue gave the expected C-1 hydroxy compound (345 mg, 179 μmol; MALDI-TOF-MS m/z = 1955 $[M + Na]^+$) in 74% yield. To a solution of this hydroxy compound (340 mg, 176 μmol) in CH_2Cl_2 (10 mL) at −30 °C was added DAST (120 μL, 880 μmol, 5 equiv) dropwise. After the solution was stirred for 2 h, the temperature of the cooling bath was raised to rt, and then the reaction was quenched by addition of MeOH and the solution was concentrated under reduced pressure. Flash chromatography (toluene/acetone, 3:1 v/v) using silica gel neutralized with triethylamine of the residue gave the expected α/β-fluorides (328 mg, 169 μmol; MALDI-TOF-MS m/z = 1957 $[M + Na]^+$) in 96% yield. In a plastic vessel, a solution of the α/β-fluorides (310 mg, 160 μmol) in hydrogen fluoride/pyridine (7:3 v/v, 3 mL) was stirred at −50 °C for 15 min, and then the temperature of the cooling bath was raised to −10 °C for 2.5 h. The solution was diluted with dichloromethane and then poured into a plastic beaker containing an ice-cooled solution of ammonia in water (3 M). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (three times), dried, and concentrated under reduced pressure. Flash chromatography (cyclohexane/acetone, 3:2 v/v) using silica gel neutralized with triethylamine of the residue gave the expected fluoride **17** (203 mg, 105 μmol) in 65% yield: $[\alpha]^{25}_D$ = +34 (c = 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) see Table E in Supporting Information; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5−168.8 (*C*OCH$_3$), 103.6 (d, $J_{C,F}$ = 229.5 Hz, C-1 of Glc^{I}), 101.4 (C-1 of Gal), 100.5, 100.3, 100.0 (C-1 of Glc^{II,III,IV}), 97.1 (C-1 of Xyl^{III}), 97.0 (C-1 of Xyl^{II}), 76.0, 75.6, 75.0, 74.7, 74.6, 74.5, 73.1, 72.7, 72.5, 72.4, 72.0, 71.9, 71.8, 71.1, 70.6, 70.6, 70.5, 70.4, 70.3, 69.5, 69.1, 69.0 (broad), 68.9, 68.6, 66.5 (C-2 to C-5 of Glc and Gal, and C-2 to C-4 of Xyl), 65.5 (C-6 of Glc^{III}), 65.1 (C-6 of Glc^{II}), 62.2 (C-6 of Glc^{IV}), 61.3 (C-6 of Glc^{I}), 60.7 (C-6 of Gal), 59.1, 58.9 (C-5 of Xyl), 20.6−20.5 (CO*C*H$_3$); MALDI-TOF-MS m/z = 1957 $[M + Na]^+$; ES-HRMS m/z calcd for $C_{80}H_{107}O_{53}FNa$ $[M + Na]^+$ 1957.5559, found 1957.5557.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(2,3,6-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-tri-*O*-acetyl-α-D-xylopyranosyl)-(1→6)]-(2,3-di-*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)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl Fluoride (18). *H. insolens* Cel7B E197A glycosynthase (5 mg) was added to a solution of lactosyl fluoride **12** (0.31 g, 0.89 mmol, 1.2 equiv) and heptasaccharide **2** (0.79 g, 0.74 mmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 15 mL). The solution was placed in a rotative shaker for 20 h at 37 °C and then freeze-dried. The residue was acetylated (acetic anhydride/pyridine, 1:2 v/v, 75 mL) in the presence of a catalytic amount of DMAP. After 12 h of stirring at rt, the reaction was quenched by addition of MeOH at 0 °C,

concentrated under reduced pressure, and coevaporated with toluene. Flash chromatography (toluene/acetone, 4:1 v/v) of the residue gave the expected acetylated nonasaccharide (1.25 g, 0.50 mmol) in 68% yield, characterized by MALDI-TOF-MS: $m/z = 2501$ $[M + Na]^+$.

The acetylated nonasaccharide was processed to give fluoride **18**, as already described for **17**. C-1 hydroxy compound was obtained from acetylated nonasaccharide (820 mg, 335 μmol) and purified by flash chromatography (toluene/acetone, 3:1 v/v) to give the expected mixture of free anomers (597 mg, 245 μmol; MALDI-TOF-MS $m/z = 2459$ $[M + Na]^+$) in 73% yield. These hydroxy compounds (464 mg, 190 μmol) were fluorinated, and the expected α/β-fluorides (443 mg, 182 μmol; MALDI-TOF-MS $m/z = 2461$ $[M + Na]^+$) were obtained in 95% yield, after flash chromatography (cyclohexane/acetone, 3:2 v/v). Anomerization of fluorides (477 mg, 195 μmol) gave, after flash chromatography (cyclohexane/acetone, 4:1, 3:1, 2:1, 3:2 then 55−45 v/v), the fluoride **18** (381 mg, 156 μmol) in 80% yield: $[\alpha]^{25}_D = +30$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.59 (dd, $J_{1,2} = 2.6$ and $J_{H,F} = 53.1$ Hz, 1H, H-1 of Glc^I), 5.01, 4.97 (m, H-1 of Xyl), 4.82, 4.76, 4.69 (m, H-1 of Glc^{III,IV,V}), 4.50 (d, $J_{1,2} = 8.0$ Hz, 1H, H-1 of Glc^{II}), 4.36 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1 of Gal), 2.09−1.91 (m, 75H, COC*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6−168.8 (*C*OCH$_3$), 103.6 (d, $J_{C,F} = 229.5$ Hz, C-1 of Glc^I), 101.4 (C-1 of Gal), 100.6, 100.4, 100.2, 100.0 (C-1 of Glc^{II,III,IV,V}), 97.1, 97.1, 96.8 (C-1 of Xyl), 75.9, 75.6, 75.3, 75.2, 74.6, 74.5 (broad), 73.2, 73.0, 72.7, 72.5, 72.4, 72.0, 72.0, 71.9, 71.5, 71.1, 70.6, 70.5, 70.5, 70.4, 70.4, 70.3, 70.3, 70.2, 69.4, 69.1, 69.1, 69.0, 68.9, 68.8, 68.5, 66.5 (C-2 to C-5 of Glc and Gal, and C-2 to C-4 of Xyl), 65.6, 65.4, 65.2 (C-6 of Glc^{II,III,IV}), 62.1, 61.2, 60.7 (C-6 of Glc^{I,V} and Gal), 59.0, 58.9, 58.9 (C-5 of Xyl), 20.9−20.2 (CO*C*H$_3$); MALDI-TOF-MS $m/z = 2461$ $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{101}H_{135}O_{67}FNa$ $[M + Na]^+$ 2461.7038, found 2461.7006.

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)-D-glucopyranose (GGXXXG 20). *O*-THP-cellobiosyl fluoride **19** (26.2 mg, 61.2 μmol, 1.3 equiv) and heptasaccharide **2** (50.0 mg, 47.0 μmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 1.5 mL) were incubated with *H. insolens* Cel7B E197A glycosynthase (2 mg) for 20 h at 37 °C. The solution was diluted with water (3.5 mL), and HCl (1M) was added to lower the pH to 1−2. After 5 mn, the solution was neutralized with Et_3N. The process was repeated twice until complete hydrolysis of the tetrahydropyranyl group. Silica gel was added and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the nonasaccharide **20** (50.6 mg, 36.5 μmol) in 78% yield: MALDI-TOF-MS $m/z = 1409$ $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{51}H_{86}O_{43}Na$ $[M + Na]^+$ 1409.4441, found 1409.4482; LC/ES-MS analysis gave *m*/*z* 1558 $[M(ABEE) + Na]^+$; $t_R = 16.47$ min.

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)-D-glucopyranose (GGGGXXXG 21). *O*-THP-cellobiosyl fluoride **19** (5.8 mg, 13.6 μmol, 1.3 equiv) and nonasaccharide **20** (14.5 mg, 10.5 μmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 1.5 mL) were incubated with *H. insolens* Cel7B E197A glycosynthase (1 mg) for 20 h at 37 °C. The solution was diluted with water (3.5 mL), and HCl (1 M) was added to lower the pH to 1−2. After 5 min, the solution was neutralized with Et_3N. The process was repeated twice until complete hydrolysis of the tetrahydropyranyl group. Silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the undecasaccharide **21** (15.3 mg, 8.9 μmol) in 86% yield: MALDI-TOF-MS $m/z = 1733$ $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{63}H_{106}O_{53}Na$ $[M + Na]^+$ 1733.5497, found 1733.5498; LC/ES-MS analysis gave *m*/*z* 1882 $[M(ABEE) + Na]^+$; $t_R = 16.09$ min.

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)-D-glucopyranose (GGGXXXG 22). *O*-THP-cellobiosyl fluoride **19** (2.7 mg, 6.4 μmol, 1.3 equiv) and octasaccharide **16** (6.0 mg, 4.9 μmol) in carbonate/bicarbonate buffer (0.1 m, pH 10, 1 mL) were incubated with *H. insolens* Cel7B E197A glycosynthase (1 mg) for 24 h at 37 °C. The solution was diluted with water (4 mL), and HCl (1 M) was added to lower the pH to 1−2. After 5 min, the solution was neutralized with Et_3N. The process was repeated twice until complete hydrolysis of the tetrahydropyranyl group. Silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the decasaccharide **22** (5.7 mg, 3.7 μmol) in 76% yield: MALDI-TOF-MS $m/z = 1571$ $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{57}H_{96}O_{48}Na$ $[M + Na]^+$ 1571.4969, found 1571.4978.

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)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XGXXXG 23). The peracetylated fluoride **14** (105 mg, 73 μmol) was treated in methanol (10 mL) with sodium methoxide (1 M in methanol, 37 μL) for 5 h at 0 °C. Sodium methoxide (37 μL) was added again, and the reaction mixture was stirred for another 12 h at 0 °C. The solution was neutralized with Amberlite IRN 120 (H^+), filtered, and concentrated under reduced pressure. The residue dissolved in deionized water was freeze-dried to give the expected pentasaccharide (56 mg, 69 μmol) in 95% yield, characterized by FAB-MS $m/z = 823$ $[M + Na]^+$.

To this free fluoride (13.2 mg, 16.5 μmol, 1.1 equiv) were added heptasaccharide **2** (15.9 mg, 15.0 μmol) in phosphate buffer (0.1 m, pH 7, 1 mL) and then *H. insolens* Cel7B E197A glycosynthase (2 mg). The solution was placed in a rotative shaker for 20 h at 37 °C, and then *H. insolens* Cel7B E197A glycosynthase (0.5 mg) was added again. After 12 h at 37 °C, recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. The reaction mixture was kept at 37 °C for 24 h, and another quantity of β-glucosidase/galactosidase (0.1 mg) was added. After 20 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the decasaccharide **23** (15.0 mg, 9.9 μmol) in 66% yield: MALDI-TOF-MS $m/z = 1541$ $[M + Na]^+$; ES-HRMS *m*/*z* calcd for $C_{56}H_{94}O_{47}Na$ $[M + Na]^+$ 1541.4863, found 1541.4857; LC/ES-MS analysis gave *m*/*z* 1690 $[M(ABEE) + Na]^+$; $t_R = 16.47$ min.

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)-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)-D-glucopyranose (XGXGXXXG 24). A mixture of deacetylated fluoride (5.2 mg, 6.4 μmol, 1.3 equiv) obtained from **14** as described for the preparation of **23**, and decasaccharide **23** (7.5 mg, 4.9 μmol) in phosphate buffer (0.1 m, pH 7, 1 mL) was incubated with *H. insolens* Cel7B E197A glycosynthase (1.5 mg). The solution was placed in a rotative shaker for 20 h at 37 °C, and recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. After 24 h at 37 °C, another quantity of β-glucosidase/galactosidase (0.1 mg) was added. After 20 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5,

9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the expected compound **24** (8.5 mg, 4.3 μmol) in 87% yield: MALDI-TOF-MS *m/z* = 1997 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{73}H_{122}O_{61}Na$ $[M + Na]^+$ 1997.6342, found 1997.6124; LC/ES-MS analysis gave *m/z* 2146 $[M(ABEE) + Na]^+$; t_R = 15.82 min.

α-D-Xylopyranosyl-(1→6)-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)-D-glucopyranose (XGGXXXG 25). A mixture of deacetylated fluoride (10.8 mg, 13.5 μmol, 1.1 equiv) obtained from **14** as described for the preparation of **23** and octasaccharide **16** (15.0 mg, 12.2 μmol) in phosphate buffer (0.1 m, pH 7, 1 mL) was incubated with *H. insolens* Cel7B E197A glycosynthase (2 mg). The solution was placed in a rotative shaker for 14 h at 37 °C, and then recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. The reaction mixture was kept at 37 °C for 24 h, and another quantity of β-glucosidase/galactosidase (0.1 mg) was added. After 24 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, 75:25, and 7:3 v/v) was used to elute the undecasaccharide **25** (15.9 mg, 9.8 μmol) in 80% yield: MALDI-TOF-MS *m/z* = 1703 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{62}H_{104}O_{52}Na$ $[M + Na]^+$ 1703.5391, found 1703.5394; LC/ES-MS analysis gave *m/z* 1852 $[M(ABEE) + Na]^+$; t_R = 16.08 min.

α-D-Xylopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-[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)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XXGXXXG 26). Compound **17** (117 mg, 60 μmol) was de-*O*-acetylated as already described for **14** to give the corresponding free fluoride (61 mg, 56 μmol) in 92% yield, characterized by MALDI-TOF-MS *m/z* = 1117 $[M + Na]^+$.

To this deprotected fluoride (15.0 mg, 13.7 μmol) were added heptasaccharide **2** (16.0 mg, 15.1 μmol, 1.1 equiv) in phosphate buffer (0.1 m, pH 7, 1.5 mL) and then *H. insolens* Cel7B E197A glycosynthase (3 mg). The solution was placed in a rotative shaker for 12 h at 37 °C, and then recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. The reaction mixture was kept at 37 °C for 24 h, and another quantity of β-glucosidase/galactosidase (0.1 mg) was added. After 24 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the undecasaccharide **26** (19.7 mg, 10.9 μmol) in 79% yield: MALDI-TOF-MS *m/z* = 1835 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{67}H_{112}O_{56}Na$ $[M + Na]^+$ 1835.5814, found 1835.5812; LC/ES-MS analysis gave *m/z* 1984 $[M(ABEE) + Na]^+$; t_R = 16.07 min.

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-(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)-D-glucopyranose (GXXGXXXG 27). A mixture of deacetylated fluoride (15.0 mg, 13.7 μmol) obtained from **17** as described for the preparation of **26** and heptasaccharide **2** (16.0 mg, 15.1 μmol, 1.1 equiv) in phosphate buffer (0.1 m, pH 7, 1.5 mL) was incubated with *H. insolens* Cel7B E197A glycosynthase (3 mg). The solution was placed in a rotative shaker for 12 h at 37 °C, and then the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, 75:25 and 7:3 v/v) was used to elute the lactosyl protected compound, which was freeze-dried. A solution of the residue in acetate buffer (0.1 m, pH 4.6, 2 mL) was treated by commercially available β-galactosidase (*A. oryzae*, 3 mg, 24 units) for 2.5 h at 30 °C. The reaction mixture was diluted in acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, and 75:25 v/v) was used to elute the expected compound **27** (9.4 mg, 4.8 μmol) in 35% yield: MALDI-TOF-MS *m/z* = 1997 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{73}H_{122}O_{61}Na$ $[M + Na]^+$ 1997.6342, found 1997.6304; LC/ES-MS analysis gave *m/z* 2146 $[M(ABEE) + Na]^+$; t_R = 15.83 min.

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-(1→4)-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XXXGGG 31). Compound **18** (200 mg, 82 μmol) was de-*O*-acetylated as already described for **14** to give the corresponding free fluoride (114 mg) in quantitative yield, characterized by MALDI-TOF-MS *m/z* = 1411 $[M + Na]^+$.

To this deprotected fluoride (20.8 mg, 15.0 μmol) were added cellobiose **28** (6.7 mg, 19.5 μmol, 1.3 equiv) in carbonate/bicarbonate buffer (0.1 m, pH 10, 1 mL) and then *H. insolens* Cel7B E197A glycosynthase (2 mg). The solution was placed in a rotative shaker for 12 h at 37 °C, and then the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, 75:25, and 7:3 v/v) was used to eluted the expected lactosyl protected nonasaccharide (19.1 mg, 11.1 μmol; MALDI-TOF-MS *m/z* = 1733 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{63}H_{106}O_{53}Na$ $[M + Na]^+$ 1733.5497, found 1733.5525; LC/ES-MS analysis gave *m/z* 1882 $[M(ABEE)^+ + Na]$; t_R = 16.29 min) in 74% yield. A solution of this undecasaccharide (17.4 mg, 10.2 μmol) in phosphate buffer (0.1 m, pH 6.8, 2 mL) was treated at 37 °C by successive additions of recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 1 mg) over 1 week. The solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the nonasaccharide **31** (10.5 mg, 7.6 μmol) in 75% yield: MALDI-TOF-MS *m/z* = 1409 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{51}H_{86}O_{43}Na$ $[M + Na]^+$ 1409.4441, found 1409.4439; LC/ES-MS analysis gave *m/z* 1558 $[M(ABEE) + 2Na]^{2+}$; t_R = 16.81 min.

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-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XXXGGGGG 32). A mixture of deacetylated fluoride (20.8 mg, 15.0 μmol) obtained from **18** as described for the preparation of **31**, and cellotetraose **29** (13.0 mg, 19.5 μmol, 1.3 equiv) in phosphate buffer (0.1 m, pH 7, 1.5 mL) were incubated with *H. insolens* Cel7B E197A glycosynthase (3 mg). The solution was placed in a rotative shaker for 12 h at 37 °C, and then recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. The reaction mixture was kept at 37 °C for 24 h, and another quantity of β-glucosidase/galactosidase (0.1 mg) was added. After 20 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, and 75:25 v/v) was used to elute the undecasaccharide **32** (12.3 mg, 7.2 μmol) in 48% yield: MALDI-TOF-MS *m/z* = 1733 $[M + Na]^+$; ES-HRMS *m/z* calcd for $C_{63}H_{106}O_{53}Na$ $[M + Na]^+$ 1733.5497,

found 1733.5411; LC/ES-MS analysis gave *m/z* 1882 [M(ABEE) + Na]$^+$; t_R = 16.33 min.

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-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XXXGXG 33). A mixture of deacetylated fluoride (21.0 mg, 15.1 μmol) obtained from **18** as described for the preparation of **31** and trisaccharide **30** (7.9 mg, 16.6 μmol, 1.1 equiv) in phosphate buffer (0.1 m, pH 7, 1.5 mL) was incubated with *H. insolens* Cel7B E197A glycosynthase (3 mg). The solution was placed in a rotative shaker for 20 h at 37 °C, and then *H. insolens* Cel7B E197A glycosynthase (0.5 mg) was added again. After 12 h at 37 °C, recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. The reaction mixture was kept at 37 °C for 24 h, and another quantity of β-glucosidase/galactosidase (0.2 mg) was added. After 20 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2 and 75:25 v/v) was used to elute the decasaccharide **33** (14.4 mg, 9.5 μmol) in 63% yield: MALDI-TOF-MS *m/z* = 1541 [M + Na]$^+$; ES-HRMS *m/z* calcd for $C_{56}H_{94}O_{47}Na$ [M + Na]$^+$ 1541.4863, found 1541.4841; LC/ES-MS analysis gave *m/z* 1690 [M(ABEE) + Na]$^+$; t_R = 16.49 min.

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-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-[α-D-xylopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→4)-D-glucopyranose (XXXGXXG 34). A mixture of deacetylated fluoride (21.0 mg, 15.1 μmol) obtained from **18** as described for the preparation of **31** and pentasaccharide **1** (12.8 mg, 16.6 μmol, 1.1 equiv) in phosphate buffer (0.1 m, pH 7, 1.5 mL) was incubated with *H. insolens* Cel7B E197A glycosynthase (3 mg). The solution was placed in a rotative shaker for 20 h at 37 °C, and then *H. insolens* Cel7B E197A glycosynthase (1 mg) was added again. After 12 h at 37 °C, recombinant β-glucosidase/galactosidase (*Agrobacterium* sp., 0.2 mg) was added. After 48 h at 37 °C, the solution was diluted with acetonitrile/water (1:1 v/v, 10 mL), silica gel was added, and the suspension was evaporated. The powder was deposited at the top of a silica gel column, and a gradient (acetonitrile then acetonitrile/water, 95:5, 9:1, 85:15, 8:2, 75:25 and 7:3 v/v) was used to elute the dodecasaccharide **34** (17.7 mg, 9.8 μmol) in 65% yield: MALDI-TOF-MS *m/z* = 1835 [M + Na]$^+$; ES-HRMS *m/z* calcd for $C_{67}H_{112}O_{56}Na$ [M + Na]$^+$: 1835.5814, found 1835.5816; LC/ES-MS analysis gave *m/z* 1984 [M(ABEE) + Na]$^+$; t_R = 16.25 min.

Enzyme Kinetics. All enzymatic reactions were done in 50 mM citrate−50 mM phosphate buffer, pH 5.5. Ionic strength was kept constant at I = 0.5 M with added KCl. Donor (1 mM) and acceptor (7 mM) were dissolved and enzyme added to a final concentration of 0.5−2 μM. Reactions were performed in a final volume of 100 μL in a thermostated bath at 30 °C. Aliquots (20 μL) were withdrawn at different time intervals and mixed with 20 μL of ManANTS 2 mM in water as internal reference, the mixture was heated at 100 °C for 10 min in a sealed tube, and finally samples were analyzed by HPCE.[35]

Capillary electrophoresis was performed on a system equipped with a diode array UV−vis detector, using a fused silica capillary, 72 cm effective length, 50 μm internal diameter with an extended light path bubble (150 μm) in the detection window. Samples (ANTS-labeled oligosaccharide standards or enzymatic reaction mixtures) were loaded into the capillary under hydrodynamic injection mode at 40 mbar pressure during 6 s. Electrophoretic separations were performed at −30 kV and constant temperature of 30 °C under inverted EOF conditions (anodic detection) using 50 mM phosphoric acid adjusted to pH 2.5 with triethylamine as running buffer. Electropherograms were recorded at 270 nm (20 nm slit).

Product concentrations from the electropherograms were determined from relative peak areas using ManANTS as internal reference. Initial rates in product formation (V_0 in mM/min) were calculated as the slope of the linear region of the time course (0−20 min) corresponding to < 10% substrate to product conversion. All kinetics were done in duplicate, and standard deviations in V_0/[E] were ≤ 5%.

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Supporting Information Available: ^{1}H NMR data for compounds **9**, **10**, **13**, **14**, and **17**; ^{1}H and ^{13}C NMR spectra for compounds **7**−**10**, **13**, **14**, **16**−**18**, **26**; quantitative HPLC-ESMS for ABEE derivatives of compounds **15**, **20**−**27**, **31**−**34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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