Synthesis of Complex Oligosaccharides by Using a Mutated (1,3)-β-D-Glucan Endohydrolase from Barley

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Abstract: Complex oligosaccharides with newly formed (1,3)-β-glycosidic linkages were obtained in good to excellent yields when substituted or unsubstituted α-laminaribiosyl fluorides, acting as donors, were condensed onto mono- and disaccharide β-D-hexopyranoside acceptors by using a (1,3)-β-D-glycosynthase. These linear and branched (1,3)-β-linked oligosaccharides could prove to be important in a range of medical, pharmaceutical, and agricultural applications. Furthermore, the observation that the (1,3)-β-D-glucan glycosynthase accommodates (1,3)-, (1,4), - and (1,6)-β-oligosaccharides in its acceptor subsites suggests novel, yet unexpected physiological roles for the wild type (1,3)-β-D-glucan endohydrolase from higher plants.

Keywords: carbohydrates · enzymatic synthesis · glycosides · glycosynthase · oligosaccharides

Introduction

(1,3)-β-D-Glucans and related polysaccharides have been implicated in immunomodulating and antitumour activities.[1, 2] They also exhibit antibacterial and antiviral properties, stimulate blood coagulation, and accelerate the healing of wounds.[3–6] In addition, (1,3)- and (1,3;1,6)-β-D-oligoglucosides elicit defense processes against microbial attack in invertebrates and plants.[7, 8] Future medical, pharmaceutical, and agricultural applications of these compounds will rely on rapid and specific synthetic procedures, that can be readily adapted to large-scale production. One approach to the rapid production of specific oligo- and polysaccharides is through "glycosynthase" technology.

Glycosynthases are generated from retaining glycoside hydrolases, in which catalytic nucleophiles are replaced with non-carboxylic amino acid residues. These mutated enzymes, which catalyze synthetic reactions without hydrolysis, are now opening new avenues for stereoselective assembly of oligosaccharides.[9–14] We have recently produced a (1,3)-β-D-glucan endohydrolase (Glu231Gly) from barley and demonstrated that the enzyme polymerizes α-laminaribiosyl fluoride under glycosynthase-like conditions.[15] In this study, we report on the versatility of this (1,3)-β-D-glycosynthase to condense α-laminaribiosyl fluoride and its derivatives with a variety of β-D-hexopyranoside acceptors.

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Results and Discussion

Table 1 (entry 1) shows the condensation of α-laminaribiosyl fluoride (**1**)[10] and *p*-nitrophenyl-D-glucoside (**2**). The identity of the newly established glycosidic linkage was confirmed by ^{13}C NMR spectroscopy with a characteristic signal at ~85 ppm that is representative of C-3 substituted β-D-glucosyl residues.[16] However, when equimolar concentrations of donor and acceptor were used, only the polymeric (1,3)-β-D-glucan product arising from the self-condensation of **1** was obtained.[15] The yield of the polymer could be minimized when the acceptor was used in a high concentration and at a donor/acceptor ratio of 1:5. Under these conditions, laminaritrioside **3** was obtained in excellent yield (90%). Reaction of the β-D-xyloside **4** and β-D-galactoside **5** gave equally good yields for the respective trisaccharide products **6** (82%) and **7** (89%) (entries 2 and 3). Under these conditions, lack of glycosylation of either *p*-nitrophenyl β-D-mannoside or 2-acetylamino-2-deoxy-β-D-glucoside suggested that both orientation and nature of the substituent at C-2 is critical for the correct location of the acceptor in subsite +1. In these last cases, only a polymeric (1,3)-β-D-glucan product was produced.

Subsequently, a series of disaccharides were used as acceptors for the condensation reactions. Entry 4 reveals that

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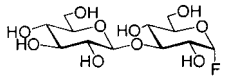
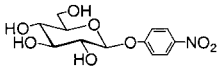
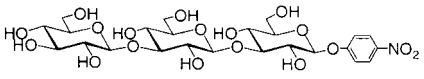
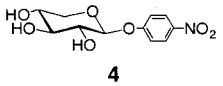
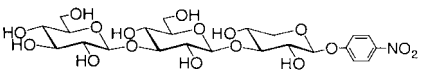
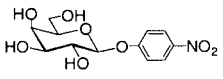
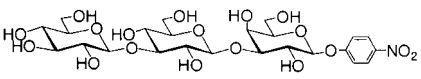
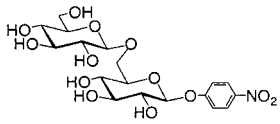
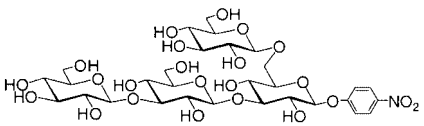
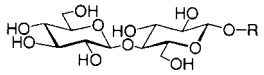
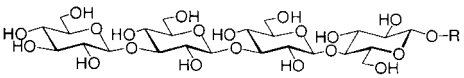
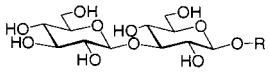
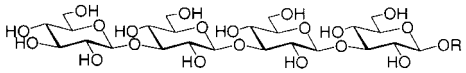
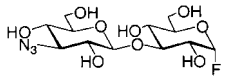
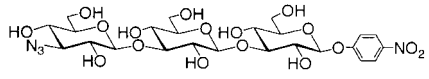
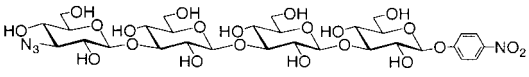
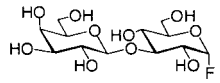
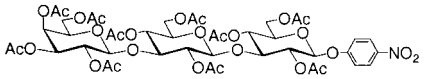
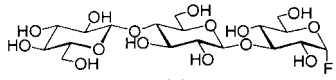
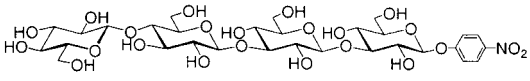
Table 1. Enzymatic syntheses of (1,3)-β-glycosidic bonds by condensation of α-laminaribiosyl fluoride **1**, and its analogues **15**, **24**, and **31**, with mono- and disaccharide acceptors **2**, **4**, **5**, **8**, **10**, **12**, and **14**, using (1,3)-β-D-glucanase Glu231Gly.

Entry	Donor	Acceptor	Isolated product	Yield
1	 **1**	 **2**	 **3**	90
2	**1**	 **4**	 **6**	82
3	**1**	 **5**	 **7**	89
4	**1**	 **8**	 **9**	70
5	**1**	 **10** R = $C_6H_4NO_2$	 **11** R = $C_6H_4NO_2$	55
6	**1**	 **12** R = Me **14** R = $C_6H_4NO_2$	 **13**	R = Me: 45 R = $C_6H_4NO_2$: 0
7	 **15**	**2**	 **22**	81
8	**15**	**14**	 **23**	90
9	 **24**	**2**	 **30**	65
10	 **31**	**2**	 **32**	33

gentiobioside **8** also serves as an acceptor in the glycosynthase reaction, and that the branched trisaccharide **9** is obtained as the sole product (70% yield). It is noteworthy that, despite having two available C(O)3 acceptor sites for glycosylation (both glucosyl units of **8** are in the β-D-configuration), only the residue harboring the aromatic aglycon is glycosylated. This result, similarly observed with a mutated β-D-mannosidase,[17] draws attention to the fact that aromatic groups play an important role in positioning the acceptor molecules in the active sites of glycosynthases. *p*-Nitrophenyl β-maltoside was not an acceptor, but *p*-nitrophenyl β-cellobioside (**10**) could be glycosylated to yield the tetrasaccharide **11** in moderate yield (55%, entry 5). It is somewhat surprising that the (1,4)-β-linked disaccharide acceptor can be correctly positioned in

the enzyme binding site, which is normally highly specific for (1,3)-β-D-glucosyl residues.[15] With the relatively short *p*-nitrophenyl β-cellobioside acceptor molecule, the glucosyl unit in the +2 subsite has to be upside down relative to the normal substrate.

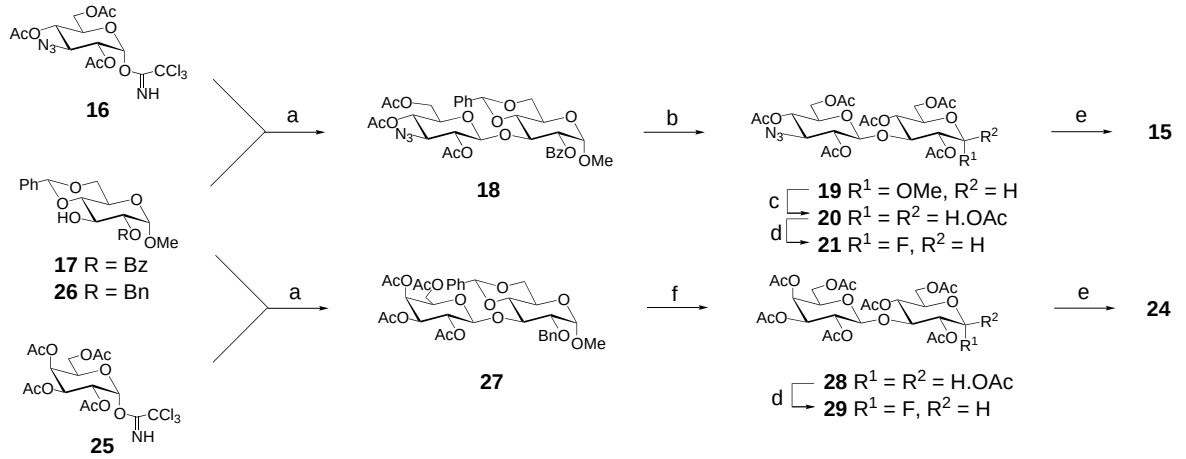
The elongation of derivatives of laminaribiose was also achieved; methyl β-laminaribioside (**12**)[18] gave the tetrasaccharide **13** in moderate yield (45%), but the *p*-nitrophenyl derivative **14**[19] yielded neither the expected product nor a (1,3)-β-D-glucan polymer (entry 6). It is tempting to speculate that this is a consequence of competition between the two disaccharides (**1** and **14**) for the glycosyl donor position in the active site. Decreasing the donor/acceptor ratio to 1:1 had no effect, and when the ratio was altered in favor of the donor **1** (5:1), the polymeric (1,3)-β-D-glucan product was synthesized.

To prevent self-condensation of the donor during equimolar coupling with acceptors, it was necessary to epimerize, protect, or substitute the 3^{II}-OH of fluoride **1**, as was observed in a (1,4)-β-D-glycosynthase.[11, 12, 20] The 3^{II}-azido fluoride **15** was therefore synthesized as outlined in Scheme 1. The trichloroacetimidate **16** was prepared in a two-step procedure from tetra-*O*-acetyl-3-azido-3-deoxy-D-glucose.[21] Glycosylation of **16** with methyl glucoside **17**[22] yielded the protected disaccharide **18** in 65% yield. After additional protective group manipulation, giving firstly the methyl glycoside (**19**) and then the heptaacetate **20**, the final fluoride (**21**) was prepared by treatment with HF in pyridine. Conventional deacetylation produced the 3^{II}-azido fluoride (**15**), which was ready for use as the glycosyl donor in the glycosynthase reaction. Thus, equimolar condensation of the 3^{II}-azido fluoride **15** with the β-D-glucoside **2** resulted in the formation of the trisaccharide **22** in excellent yield (81%) (Table 1 entry 7). This result opens the route to an efficient synthesis of bifunctionalized (1,3)-β-D-oligosaccharides for the sensitive assay of (1,3)-β-D-glucan endohydrolases by fluorescence quenching, as already reported cellulases.[20] However, a similar ratio could not be employed for the synthesis involving the disaccharide acceptor **14**, because of its inherent inhibitory activity. In this case, a fivefold excess of donor was required to drive the synthetic reaction, producing the tetrasaccharide **23** in near quantitative yield (entry 8).

To further investigate the versatility of the barley (1,3)-β-D-glycosynthase, α-D-glucopyranosyl, α-lactosyl, and α-cellobiosyl fluorides were tried as glycosyl donors. The failure of these glycosides to condense onto glucoside **2** is in accordance with the tight substrate specificity of the wild-type enzyme. Therefore, the (1,3)-β-D-glycosynthase requires at least a (1,3)-β-D-linked disaccharide motif in its glycosyl donor position (subsites −1 and −2).[23] Having observed a tolerance of the barley (1,3)-β-D-glycosynthase for a substituted C-3^{II} OH group on the donor molecule, we investigated the possibility that a donor with an axial OH group at C-4^{II} would be viable in the synthetic reaction. The synthesis of the C-4^{II} modified disaccharide **24** is outlined in Scheme 1. Coupling of imidate **25**[24] with the methyl glycoside (**26**)[25] gave disaccharide **27** (85% yield), and subsequently, octaacetate **28** and fluoride **29** were prepared prior to deacetylation. Condensation of the donor **24** with the β-D-glucoside **2** and subsequent acetylation of the reaction products gave rise to trisaccharide **30** in 65% yield (Table 1 entry 9). As observed for the cellooligosaccharide series,[26] the (1,3)-β-D-oligosaccharide with a terminal β-D-galactosyl residue could prove to be a useful compound for oligosaccharide modification by the galactose–oxidase reaction.

Finally, we investigated the versatility of the trisaccharide fluoride **31**, which arises from the final hydrolysis product of cellulase-mediated hydrolysis of (1,3;1,4)-β-D-glucans.[27] The trisacchaaride was modified by acetylation, fluorination, and *trans*-esterification. Entry 10 reveals that while the synthesis of tetrasaccharide **32** was achieved, its low yield (33%) suggested that the cellobiosyl unit was not well positioned in the donor subsites −3 and −2.

In summary, the barley (1,3)-β-D-glycosynthase was used for the specific synthesis of complex oligosaccharides, including (1,3)-β-D-oligoglucosides, C-6 substituted (1,3)-β-D-oligoglucosides, (1,3)-β-D-glucoxylosides, (1,3)-β-D-galactoglucosides, and linear (1,3;1,4)-β-D-oligoglucosides. While the (1,3)-β-D-glycosynthase activity depends on forming produc-



Scheme 1. Syntheses of modified laminaribiosyl fluorides **15** and **24**. a) TMSOTf, CH_2Cl_2; b) 1) NaOMe, MeOH, 2) 60% AcOH, 100 °C, 3) Ac_2O, pyridine, *N,N*-dimethylaminopyridine; c) H_2SO_4, AcOH, Ac_2O (1:50:100 v/v; d) HF/pyridine; e) NaOMe, MeOH; f) (1) H_2, Pd/C, MeOH, EtOAc, AcOH, 2) H_2SO_4, AcOH, Ac_2O (1:25:50 v/v).

tive complexes at subsites −2 and −1 with α-laminaribiosyl fluorides, it displays a greater tolerance for unusual acceptor molecules at subsites +1, +2, and beyond. The range and complexity of oligo- and polysaccharides that are synthesized by individual glycosynthases could be extended considerably by the sequential use of the various glycosynthases that are now available.[28, 29]

Experimental Section

Melting points were measured by using a Buchi 535 melting point apparatus and remain uncorrected. Optical rotations were measured by means of a Perkin–Elmer 341 polarimeter (volume of microcell 1 mL, 10 cm path length) at room temperature. ^{1}H and ^{13}C NMR spectra were recorded by using a Bruker AC-300 (300 MHz for ^{1}H and 75.5 MHz for ^{13}C) or a Varian Unity 500 spectrometer (500 MHz for ^{1}H and 125.8 MHz for ^{13}C) either in deuterium oxide (D_2O), employing residual H_2O (^{1}H, δ = 4.78) as internal standard, or in deuteriochloroform ($CDCl_3$) with residual $CHCl_3$ (^{1}H, δ7.26) and $CDCl_3$ (^{13}C, δ = 77.0) being employed as internal standards, at ambient temperatures (298 K) unless specified otherwise. Where appropriate, analysis of NMR spectra was aided by COSY (correlated spectroscopy) experiments. High resolution mass spectra (HRMS) were recorded by using VG ZAB and low resolution MS with a Nermag R-1010C spectrometer by using the fast atom bombardment (FAB) technique and employing NaCl as the matrix. The *m/z* data for the peaks correspond to [M^++Na]. Microanalyses were performed by the "Laboratoire Central d'analyses du CNRS" (Vernaisson, France). All syntheses that involved anhydrous solvents were performed under argon. Flash chromatography was performed on Merck (Darmstadt, Germany) silica gel (40–63 μm) under a positive pressure with the specified eluents. Reversed-phase chromatography was performed on C18 Sep-Pak cartridges (Waters, USA) under a positive pressure by using 5–20% (v/v) MeOH/H_2O as eluent. Progress of the reactions were monitored by thin-layer chromatography (TLC) with commercially prepared Merck silica gel 60 F_{254} aluminium plates. Compounds detected on TLC plates were visualized by charring with 5% (v/v) sulfuric acid in methanol and/or under ultraviolet light. The term "work-up" refers to dilution with water, extraction into an organic solvent, sequential washing of the organic extract with aqueous hydrochloric acid (1M, where appropriate), saturated aqueous sodium bicarbonate and brine, followed by drying over anhydrous magnesium sulfate, filtration, evaporation of the solvent by means of a rotary evaporator at reduced pressure, and drying of the residue at 1 mm Hg. *p*-Nitrophenyl glycosides of β-D-glucose, β-D-xylose, β-D-galactose, β-D-mannose, 2-acetylamino-2-deoxy-β-D-glucose, β-cellobiose, and β-gentiobiose were obtained from Sigma Chemical Co. (St. Louis, USA).

General procedure for enzymatic synthesis catalyzed by the glycosynthase: The mutated (1,3)-β-D-glucan endohydrolase Glu231Gly (final concentration of 0.1 mg mL^{-1}) was added to a solution of the fluoride donor (1.0 equiv of 10 mM), and the acceptor (5.0 equiv of 10 mM) was dissolved in the phosphate buffer (0.25 M, pH 7.0). The combined mixture was incubated at 37 °C for 12–24 h. The reaction products were separated directly from the reaction mixture by reversed-phase chromatography. Evaporation and/or lyophilisation of the solvents first gave the unreacted acceptor >(4.0 equiv), and subsequently the product of the transglycosylation reaction.

***p*-Nitrophenyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-β-D-glucopyranoside (3)**: Condensation of α-laminaribiosyl fluoride (**1**)[10] and 4-nitrophenyl β-D-glucoside (**2**) gave trisaccharide **3** as an amorphous powder (90%); ^{1}H NMR (D_2O): δ = 8.16–8.12, 7.26–7.22 (2m, 4H, ArH), 5.20 (d, J(1,2) = 7.3 Hz, 1H; H-1^{I}), 4.74, 4.66 (2 d, J(1,2) = J(1,2) = 8.0 Hz, 2H; H-1^{II,III}), 3.88-3.24 ppm (m, 18H; H-2$^{I–III}$, H-3$^{I–III}$, H-4$^{I–III}$, H-5$^{I–III}$, H-6$^{I–III}$); ^{13}C NMR (D_2O) δ = 162.04, 143.02 (2C; ArC), 126.47, 116.88 (4 C; ArCH), 103.16, 102.87, 99.63 (C-1$^{I–III}$), 84.66, 84.11 (C-3^{I,II}), 76.36, 76.28, 75.99, 75.92, 73.83, 73.59, 72.92, 69.94, 68.51, 68.17 (C-2$^{I–III}$, C-3^{III}, C-4$^{I–III}$, C-5$^{I–III}$), 61.07, 60.79 ppm (3C; C-6$^{I–III}$); HRMS-FAB: *m/z* calcd for $C_{24}H_{35}NO_{18}Na$: 664.1523; found: 664.1525.

***p*-Nitrophenyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-β-D-xylopyranoside (6)**: Condensation of α-laminaribiosyl fluoride (**1**) and 4-nitrophenyl β-D-xyloside (**4**) yielded the trisaccharide **6** as an amorphous powder (82%); ^{1}H NMR (D_2O): δ = 8.24–8.18, 7.24–7.18 (2m, 4H; ArH), 5.20 (d, J(1,2) = 6.9 Hz, 1H; H-1^{I}), 4.79, 4.69 (2d, J(1,2) = J(1,2) = 8.0 Hz, 2H; H-1^{II,III}), 4.07–4.01, 3.90–3.62, 3.56–3.24 ppm (3m, 18H; H-2$^{I–III}$, H-3$^{I–III}$, H-4$^{I–III}$, H-5$^{I–III}$, H-6$^{I–III}$); ^{13}C NMR (D_2O): δ = 163.14, 144.31 (2C; ArC), 127.71, 118.13 (4C; ArCH), 104.41, 103.99, 101.43 (C-1$^{I–III}$), 85.92, 84.86 (C-3^{I,II}), 77.61, 77.22, 77.18, 75.06, 74.80, 73.88, 71.18, 69.78, 69.16 (C-2$^{I–III}$, C-3^{III}, C-4$^{I–III}$, C-5^{II,III}), 66.54 (C-5^{I}), 61.30 ppm (3C; C-6$^{I–III}$); MS-FAB: *m/z*: 618 [M^++Na].

A sample of the trisaccharide was treated with Ac_2O/pyridine/*N*,*N*-dimethylaminopyridine (12 h) and then subjected to workup ($CHCl_3$) and flash chromatography (60–70% EtOAc/petrol ether) to yield *p*-nitrophenyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4-di-*O*-acetyl-β-D-xylopyranoside as a colorless oil; $[\alpha]_D$ = −23.0° (c = 0.4 in $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ = 8.22–8.17, 7.18–7.12 (2m, 4H, ArH), 5.45–5.43 (br d, J(1,2) = 2.6 Hz, 1H; H-1^{I}), 5.13 (t, J(2,3) = J(3,4) = 9.3 Hz, 1H; H-3^{III}), 5.05 (dd, J(1,2) = 8.0, J(2,3) = 9.7 Hz, 1H; H-2^{II}), 5.04 (t, J(3,4) = J(4,5) = 9.3 Hz, 1H; H-4^{III}), 4.99–4.91 (m, 2H, H-2^{I},H-4^{I}), 4.88 (dd, J(1,2) = 8.2 Hz, J(2,3) = 9.3 Hz, 1H; H-2^{III}), 4.57, 4.56 (2d, J(1,2) = J(1,2) = 8.0 Hz, 2H; H-1^{II,III}), 4.36 (dd, J(5,6) = 4.4 Hz, J(6,6) = 12.4 Hz, 1H; H-6^{II}), 4.22 (dd, J(5,6) = 4.9, J(6,6) = 12.4 Hz, 1H; H-6^{III}), 4.17–4.10 (m, 2H; H-5^{I},H-6^{II}), 4.03 (dd, J(5,6) = 2.2 Hz, J(6,6) = 12.4 Hz, 1H; H-6^{III}), 3.95–3.91 (m, 1H; H-3^{I}), 3.87 (t, J(2,3) = J(3,4) = 9.5 Hz, 1H; H-3^{II}), 3.73–3.60 (m, 3H; H-5$^{I–III}$), 2.14–1.97 ppm (9 s, 27H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): δ = 170.68, 170.46, 170.33, 169.67, 169.35, 169.27, 169.14, 168.69 (9 C; C=O), 160.82, 142.87 (2 C; ArC), 125.77, 116.56 (4 C; ArCH), 101.99, 100.99, 99.61 (C-1$^{I–III}$), 78.74 (C-3^{II}), 74.16, 72.93, 72.54, 72.12, 71.78, 71.78, 71.11, 68.97, 68.20, 68.12, 67.72 (C-2$^{I–III}$, C-3^{I,III}, C-4$^{I–III}$, C-5^{II,III}), 62.02, 61.75 (C-6^{II,III}), 59.59 (C-5^{I}), 20.87, 20.83, 20.67, 20.62, 20.51, 20.46, 20.39 ppm (9 C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{41}H_{51}NO_{26}Na$: 996.2597; found: 996.2597.

***p*-Nitrophenyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside (7)**: Condensation of α-laminaribiosyl fluoride (**1**) and *p*-nitrophenyl β-D-galactoside (**5**) yielded the trisaccharide (**7**) as an amorphous powder (89%); ^{1}H NMR (D_2O) δ = 8.26–8.18, 7.20–7.18 (2m, 4H; ArH), 5.20 (d, J(1,2) = 7.3 Hz, 1H; H-1^{I}), 4.70 (2d, J(1,2) = J(1,2) = 8.0 Hz, 1H; H-1^{II,III}), 4.22 (d, J(3,4) = 3.0 Hz, J(4,5) = 0.8 Hz, 1H; H-4^{I}), 4.00–3.82, 3.75–3.62, 3.57–3.25 ppm (3m, 17H; H-2$^{I–III}$, H-3$^{I–III}$, H-4^{II,III}, H-5$^{I–III}$, H-6$^{I–III}$); ^{13}C NMR (D_2O): δ = 162.07, 142.96 (2C; ArC), 126.43, 116.83 (4C; ArCH), 103.84, 103.13, 100.06 (C-1$^{I–III}$), 85.58, 82.87 (C-3^{I,II}), 76.34, 75.89, 75.78, 75.67, 73.79, 73.42, 69.91, 69.83, 68.41, 68.27 (C-2$^{I–III}$, C-3^{III}, >C-4$^{I–III}$, C-5$^{I–III}$), 61.04, 60.96, 60.84 ppm (C-6$^{I–III}$); MS-FAB: *m/z*: 648 [M^++Na].

A sample of the trisaccharide was treated with Ac_2O/pyridine/*N*,*N*-dimethylaminopyridine (12 h) and then subjected to workup ($CHCl_3$) and flash chromatography (65–75% EtOAc/petrol ether) to yield *p*-nitrophenyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside as a colorless oil; $[\alpha]_D$ −11.9° (c = 0.8 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 8.21–8.14, 7.06–7.01 (2m, 4H; ArH), 5.42 (dd, J(1,2) = 8.0 Hz, J(2,3) = 10.0 Hz, 1H; H-2^{III}), 5.42–5.40 (m, 1H; H-4^{I}), 5.11 (t, J(2,3) = J(3,4) = 9.3 Hz, 1H; H-3^{III}), 5.03 (t, J(3,4) = J(4,5) = 9.5 Hz, 1H; H-4^{III}), 5.03 (d, J(1,2) = 7.9 Hz, 1H; H-1^{I}), 4.96–4.83 (m, 3H; H-2^{I,II}, H-4^{II}), 4.53, 4.45 (2d, J(1,2) = J(1,2) = 8.0 Hz, 2H; H-1^{II,III}), 4.39 (dd, J(5,6) = 2.9 Hz, J(6,6) = 12.0 Hz, 1H; H-6^{I}), 4.35 (dd, J(5,6) = 4.0 Hz, J(6,6) = 12.2 Hz, 1H; H-6^{II}), 4.14–3.88, 3.87–3.62, 3.69–3.60 (3m, 9H; H-3^{I,II}, H-5$^{I–III}$, H-6$^{I–III}$), 2.12, 2.11, 2.10, 2.08, 2.05, 2.04, 2.01, 1.99, 1.98, 1.95 ppm (10 s, 30H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): δ = 170.86, 170.46, 170.43, 170.31, 169.72, 169.35, 169.22, 169.12, 168.69, 169.41 (10 C; C=O), 161.33, 143.17 (2 C; ArC), 125.71, 116.54 (4 C; ArCH), 100.96, 100.72, 99.36 (C-1$^{I–III}$), 78.37 (C-3^{II}), 75.61, 72.87, 72.56, 71.95, 71.90, 71.63, 71.01, 70.19, 68.49, 68.31, 68.14 (C-2$^{I–III}$, C-3^{I,III}, C-4$^{I–III}$, C-5$^{I–III}$), 62.04, 61.74, 61.07 (C-6$^{I–III}$), 20.82, 20.78, 20.66, 20.61, 20.50, 20.33 ppm (10C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{44}H_{55}NO_{28}Na$: 1068.2808; found: 1068.2813.

***p*-Nitrophenyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (9)**: Condensation of α-laminaribiosyl fluoride (**1**) and *p*-nitrophenyl β-gentiobioside (**8**) yielded the trisaccharide (**9**) as an amorphous powder (70%); ^{1}H NMR (D_2O) δ = 8.25–8.19, 7.26–7.20 (2m, 4H; ArH), 5.27 (d, J(1,2) = 7.3 Hz, 1H; H-1^{I}), 4.78, 4.69 (2d, J(1,2) = 8.0 Hz, J(1,2) = 7.7 Hz, 2H; H-1^{II,III}), 4.41 (d, J(1,2) = 7.7 Hz, 1H; H-1^{IV}), 4.20–4.14, 3.91–3.58, 3.55–3.19 ppm (3m,

24H; H-2^{I-IV}, H-3^{I-IV}, H-4^{I-IV}, H-5^{I-IV}, H-6^{I-IV}); ^{13}C NMR (D_2O): $\delta = 161.95$, 143.10 (2C; ArC), 126.48, 116.99 (4C; ArCH), 103.16, 102.86, 99.51 (4C; C-1^{I-IV}), 84.67, 83.39 (C-3^{I,II}), 76.37, 76.24, 76.02, 75.50, 73.83, 73.60, 73.48, 72.89, 69.98, 68.80, 68.51, 68.12 (15C; C-2^{I-IV}, C-3^{III,IV}, C-4^{I-IV}, C-5^{I-IV}, C-6^{I}), 61.07 ppm (3C; C-6^{II-IV}); MS-FAB: *m/z*: 810 [M^++Na].

A sample of this trisaccharide was treated with Ac_2O/pyridine/*N,N*-dimethylaminopyridine (12 h) and then subjected to workup ($CHCl_3$) and flash chromatography (65–75% EtOAc/petrol ether) to yield *p*-nitrophenyl (2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)-(1→3)-[2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-*β*-D-glucopyranoside as a colorless oil ; $[\alpha]_D = -32.3^\circ$ (*c* = 1.1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.29-8.18$, 7.05–6.19 (2m, 4H; ArH), 5.22–4.75 (m, 11H; H-1^{I}, H-2^{I-IV}, H-3^{III,IV}, H-4^{I-IV}), 4.49, 4.48, 4.46 (3d, $J(1,2) = J(1,2) = 7.7$ Hz, $J(1,2) = 7.8$ Hz, 3H; H-1^{II-IV}), 4.39–4.19, 4.15–3.75 (m, 10H; H-3^{I,II}, H-6^{I-IV}), 3.66–3.53 (m, 4H; H-5^{I-IV}), 2.13, 2.09, 2.06, 2.05, 2.04, 2.00, 1.99, 1.97, 1.95 ppm (13s, 39H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.48$, 170.43, 170.25, 170.09, 169.41, 169.38, 169.22, 169.15, 169.09, 168.74, 168.68 (13C; C=O), 160.82, 143.26 (2C; ArC), 126.09, 116.42 (4C; ArCH), 101.05, 100.61, 98.16 (4C; C-1^{I-IV}), 78.90, 77.73 (C-3^{I,II}), 74.09, 72.84, 72.61, 72.48, 72.05, 71.85, 71.65, 71.07, 70.92, 68.48, 68.29, 68.19, 68.09 (17C; C-2^{I-IV}, C-3^{III,IV}, C-4^{I-IV}, C-5^{I-IV}, C-6^{I}), 61.99, 61.71, 61.69 (C-6^{II-IV}), 20.90, 20.68, 20.58, 20.52, 20.49, 20.39, 20.34 ppm (13C; C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{56}H_{71}NO_{36}Na$: 1333.3756; found: 1333.3659.

p-Nitrophenyl (β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→4)-β-D-glucopyranoside (11): Condensation of *α*-laminaribiosyl fluoride (**1**) and *p*-nitrophenyl *β*-cellobioside (**10**) yielded the tetrasaccharide **11** as an amorphous powder (55%); ^{1}H NMR (D_2O): $\delta = 8.24-8.18$, 7.24–7.18 (2m, 4H; ArH), 5.23 (d, $J(1,2) = 7.7$ Hz, 1H; H-1^{I}), 4.72, 4.68 (2d, $J(1,2) = 8.0$ Hz, $J(1,2) = 8.2$ Hz, 2H; H-1^{III,IV}), 4.68 (d, $J(1,2) = 8.0$ Hz, 1H; H-1^{II}), 3.99–3.62, 3.55–3.27 ppm (2m, 24H; H-2^{I-IV}, H-3^{I-IV}, H-4^{I-IV}, H-5^{I-IV}, H-6^{I-IV}); ^{13}C NMR (D_2O): $\delta = 163.28$, 144.28 (2C; ArC), 127.73, 118.13 (4C; ArCH), 104.42, 104.10, 103.95, 100.89 (C-1^{I-IV}), 85.90, 85.11 (2C; C-3^{II,III}), 79.81 (C-4^{I}), 77.62, 77.23, 77.18, 76.71, 75.62, 75.07, 74.83, 74.64, 74.15, 71.19, 62.31, 62.20, 61.39 (C-2^{I-IV}, C-3^{I,IV}, C-4^{II-IV}, C-5^{I-IV}), 62.31, 62.20, 61.39 ppm (4C; C-6^{I-IV}).

A small sample of the tetrasaccharide was treated with Ac_2O/pyridine/*N,N*-dimethylaminopyridine (12 h) and then subjected to workup ($CHCl_3$) and flash chromatography (75% EtOAc/petrol ether) to yield *p*-nitrophenyl (2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside as a colorless oil; $[\alpha]_D = -30.0^\circ$ (*c* = 0.7 in $CHCl_3$); ^{13}C NMR ($CDCl_3$) $\delta = 170.55$, 170.47, 170.33, 170.29, 169.65, 169.41, 169.24, 169.08, 169.03, 168.73, 168.38 (13C; C=O), 161.18, 143.29 (2C; ArC), 125.85, 116.58 (4C; ArCH), 101.06, 100.74, 100.64, 97.91 (4C; C-1^{I-IV}), 78.98, 77.88 (C-3^{II,III}), 75.84, 73.32, 73.01, 72.87, 72.60, 72.34, 72.12, 71.82, 71.66, 71.07, 70.88, 68.11, 68.00 (14C; C-2^{I-IV}, C-3^{I,IV}, C-4^{I-IV}, C-5^{I-IV}), 62.02, 61.94, 61.72 (4C; C-6^{I-IV}), 58.45 (OMe), 20.85, 20.74, 20.68, 20.68, 20.50, 20.49, 20.47, 20.43, 20.33 ppm (13C; C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{56}H_{71}NO_{36}Na$: 1356.3653; found: 1356.3638.

Methyl (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-acetyl-β-D-glucopyranoside (13): *α*-Laminaribiosyl fluoride (**1**) and methyl *β*-laminaribioside (**12**)[18] were subjected to the glycosynthase reaction (24 h) and the solution was then lyophilized. The residue was treated with Ac_2O (2mL), pyridine (3mL), and *N,N*-dimethylaminopyridine (100 mg), and the combined mixture stirred (RT 12 h). The mixture was subjected to workup ($CHCl_3$) and flash chromatography (60–80% EtOAc/petrol ether) to yield the tetrasaccharide (**13**) as a colorless oil (45%); $[\alpha]_D = -23.2^\circ$ (*c* = 0.7 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.12-4.76$ (m, 9H; H-2^{I-IV}, H-3^{IV}, H-4^{I-IV}), 4.48, 4.46 (2d, $J(1,2) = 8.0$ Hz, $J(1,2) = 8.2$ Hz, 2H; H-1^{II,III}), 4.39–4.23, 4.18–3.98 (2m, 10H; H-1^{I,IV}, H-6^{I-IV}), 3.85, 3.78, 3.77 (3t, $J(2,3) = J(3,4)$ 9.1 Hz, 3H; H3^{I-III}), 3.70–3.62 (m, 4H; H-5^{I-IV}), 3.39 (s, 3H; OMe), 2.12, 2.09, 2.06, 2.05, 2.04, 2.01, 2.00, 1.99, 1.98, 1.95 ppm (13s, 39H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.73$, 170.60, 170.45, 170.22, 169.45, 169.26, 169.18, 169.09, 169.03, 168.88, 168.81 (13C; C=O), 101.45, 101.04, 100.76, 100.49 (C-1^{I-IV}), 78.86, 78.22, 78.16 (C-3^{I-III}), 72.51, 71.88, 71.63, 70.87, 68.43, 68.27, 68.07 (13C; C-2^{I-IV}, C-3^{IV}, C-4^{I-IV}, C-5^{I-IV}), 62.22, 62.13, 61.97, 61.71 (C-6^{I-IV}), 56.62 (OMe), 21.07, 20.80, 20.75, 20.69, 20.60, 20.51, 230.49, 20.34 ppm (13C; C=OCH_3); MS-FAB: *m/z*: 1249 [M^++Na].

2,3,6-Tri-O-acetyl-3-azido-3-deoxy-α-D-glucopyranosyl trichloroacetimidate (16): i) Hydrazine acetate (385 mg, 4.2 mmol) was added to a warmed (50°C) solution of 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-glucose[21] (1.42 g, 3.8 mmol) in DMF (20mL), and the combined mixture was stirred for 30 min. The mixture was poured onto saturated NaCl solution and extracted (EtOAc). The combined extracts were dried ($MgSO_4$), filtered, and evaporated, and the residual oil subjected to flash chromatography (40–45% EtOAc/petrol ether) to yield 2,3,6-tri-*O*-acetyl-3-azido-3-deoxy-D-glucopyranose as a colorless oil (1.45 g, 91%; *α*/*β*1:1); *α*-anomer: ^{13}C NMR ($CDCl_3$) $\delta = 170.95$, 170.04, 169.43 (C=O), 89.50 (C-1), 71.88, 68.45, 67.25 (C-2, C-4, C-5), 62.03 (C-6), 60.57, (C-3), 20.64, 20.57, 20.54 ppm (Me); *β*-anomer: ^{13}C NMR ($CDCl_3$): $\delta = 170.94$, 170.56, 169.31 (C=O), 95.64 (C-1), 73.34, 72.84, 63.85 (C-2, C-4, C-5), 62.03 (C-6), 60.46, (C-3), 20.94, 20.64, 20.57 (C=OCH_3); MS-FAB: *m/z*: 349 [M^++NH_4].

ii) DBU (50 μL) was added to a solution of the previously described product (1.1 g, 3.3 mmol) and trichloroacetonitrile (1.0mL, 10 mmol) in CH_2Cl_2 (25mL), and the combined mixture stirred (0°C→RT, 2 h). The mixture was evaporated, and the residual oil subjected to flash chromatography (10% EtOAc/petrol ether) to yield the trichloroacetimidate **16** as a colorless oil (1.30 g, 81%); ^{1}H NMR ($CDCl_3$): $\delta = 8.68$ (br s, 1H; NH), 6.49 (d, $J(1,2) = 3.5$ Hz, 1H; H-1), 5.04 (t, $J(3,4) = 10.0$ Hz, 1H; H-4), 4.96 (dd, $J(1,2) = 3.5$ Hz, $J(2,3) = 10.6$ Hz, 1H; H-2), 4.22–3.99 (m, 4H; H-3, H-5, H-6), 2.11, 2.06, 2.04 ppm (3s, C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta =$ 169.51, 169.09 (3C; C=O), 160.56 (C=NH), 92.51 (2C; C-1, CCl_3), 70.45, 70.22, 67.67 (C-2, C-4, C-5), 61.40, 60.95 (C-3, C-6), 20.60, 20.57, 20.94 (C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{14}H_{17}Cl_3N_4O_8Na$: 497.0010; found: 497.0005.

Methyl (2,4,6-tri-O-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)-(1→3)-2-O-benzoyl-4,6-O-benzylidine-α-D-glucopyranoside (18): Freshly activated molecular sieves (powdered 4 Å, 500 mg) were added to a solution of azide **16** (2.17 g, 4.6 mmol) and alcohol **17**[22] (881 mg, 2.3 mmol) in CH_2Cl_2 (25mL), and the combined mixture stirred (RT, 15 min). TMSOTf (50 μL) was introduced with continued stirring (10 min) followed by Et_3N (200 μL). The mixture was filtered and evaporated and the residue subjected to flash chromatography (30–35% EtOAc/petrol ether) to yield the title compound as a colorless foam (1.04 g, 65%); $[\alpha]_D + 36.0^\circ$ (*c* = 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.09-8.05$, 7.65–7.60, 7.54–7.45, 7.34–7.29 (m, 10H; ArH), 5.11 (dd, $J(1,2) = 3.8$ Hz, $J(2,3) = 9.5$ Hz, 1H; H-2^{I}), 4.99 (dd, $J(1,2) = 3.8$ Hz, 1H; H-1^{I}), 4.92 (t, $J(4,5) = 7.8$, $J(3,4) = 9.9$ Hz, 1H; H-4^{II}), 4.87 (dd, $J(1,2) = 7.8$ Hz, $J(2,3) = 9.9$ Hz, 1H; H-2^{II}), 4.69 (d, $J(1,2) =$ 7.8, 1H; H-1^{II}), 4.34 (t, $J(3,4) = J(4,5) = 9.3$ Hz, 1H; H-4^{I}), 4.27 (d, $J(5,6) =$ 4.0 Hz, $J(6,6) = 9.5$ Hz, 1H; H-6^{I}), 4.14 (dd, $J(5,6) = 4.9$ Hz, $J(6,6) =$ 12.3 Hz, 1H; H-6^{II}), 3.99 (dd, $J(5,6) = 2.6$ Hz, $J(6,6) = 12.2$ Hz, 1H; H-6^{II}), 3.95–3.65 (m, 4H; H-3^{I}, H-5^{I}, H-6^{I}, PhC*H*), 3.54 (ddd, $J(5,6) =$ 2.7 Hz, $J(5,6) = 4.6$ Hz, $J(4,5) = 9.9$ Hz, 1H; H-5^{II}), 3.36 (s, 3H; OMe), 3.20 (t, $J(2,3) = J(3,4) = 9.9$ Hz, 1H; H-3^{II}), 2.05, 1.95, 1.67 (3s, 9H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.73$, 169.03, 168.91 (C=OCH_3), 165.61 (*C*= OAr), 137.25, 133.60, 129.80, 129.40, 128.96, 128.58, 128.04, 126.03 (12C; Ar), 101.14, 101.03 (C-1^{II}, Ph*C*H), 97.59 (C-1^{I}), 79.05 (C-3^{I}), 76.29, 73.62, 72.47, 70.75, 68.76, 68.28 (C-2^{I,II}, C-4^{I,II}, C-5^{I,II}), 64.37 (C-3^{II}), 62.57, 62.06 (C-6^{I,II}), 55.38 (OMe), 20.63, 20.54, 19.98 ppm (C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{33}H_{37}N_3O_{14}Na$ 722.2173; found: 722.2179.

Methyl (2,4,6-tri-O-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-acetyl-α-D-glucopyranoside (19): NaOMe (3mL of 1.0M in MeOH, 3.0 mmol) was added to a solution of the azide (**18**) (1.40 g, 2.00 mmol) in MeOH (15mL), and the mixture stirred (0°C→RT, 2 h). The solution was treated with Amberlite IR-120 resin (H^+ form) until neutral and filtered; the solvent was then evaporated. Acetic acid (20mL of 60% aq.) was added to the residual oil, and the combined mixture heated (100°C; 20 min) and then evaporated (and co-evaporated with toluene). Ac_2O (6mL), pyridine (8mL), and *N,N*-dimethylaminopyridine (50 mg) were added to the residual oil, and the combined mixture was stirred (RT, 8 h) before being treated with ice-water (10mL). The mixture was then subjected to workup (CH_2Cl_2) and flash chromatography (45–50% EtOAc/petrol ether) yielding the hexaacetate (**19**) as fine needles (1.10 g, 87%); m.p. 153–154°C (EtOH); $[\alpha]_D = +21.9^\circ$ (*c* = 1.0 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 4.94$ (dd, $J(1,2) = 3.6$ Hz, $J(2,3) = 10.2$ Hz, 1H; H-2^{I}), 4.93-4.81 (m, 3H; H-1^{I}, H-4^{I,II}), 4.77 (dd, $J(1,2) = 8.0$ Hz, $J(2,3) =$ 10.0 Hz, 1H; H-2^{II}), 4.57 (d, $J(1,2) = 8.0$ Hz, 1H; H-1^{II}), 4.25 (dd, $J(5,6) =$ 4.6 Hz, $J(6,6) = 12.4$ Hz, 1H; H-6^{II}), 4.18–4.06 (m, 3H; H-3^{I}, H-6^{I,II}), 4.00 (dd, $J(5,6) = 2.4$ Hz, $J(6,6) = 12.2$ Hz, 1H; H-6^{II}), 3.87 (ddd, $J(5,6) = 2.6$ Hz,

$J(5,6) = 4.6$ Hz, $J(4,5) = 10.4$ Hz, 1H; H-5^{I}), 3.58 (ddd, $J(5,6) = 2.4$ Hz, $J(5,6) = 4.6$ Hz, $J(4,5) = 9.8$ Hz, 1H; H-5^{II}), 3.48 (t, $J(2,3) = J(3,4) = 10.0$ Hz, 1H; H-3^{II}), 3.66 (s, 3H, OMe), 2.15, 2.06, 2.05, 2.03, 2.02, 1.98 ppm (6s, 18H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.63$, 170.43, 169.59, 169.13, 169.04, 168.68 (6C; C=O), 100.91 (C-1^{II}), 96.67 (C-1^{I}), 76.09 (C-3^{I}), 72.77, 72.49, 70.84, 68.18, 67.99, 67.33 (C-2^{I,II}, C-4^{I,II}, C-5^{I,II}), 64.39 (C-3^{II}), 62.08, 61.75 (C-6^{I,II}), 55.34 (OMe), 20.87, 20.66, 20.56, 20.51, 20.38, 20.31 ppm (6C; C=O*C*H$_3$); MS-FAB: *m/z*: 656 [M^++Na]; elemental analysis calcd (%) for $C_{25}H_{35}N_3O_{16}$ (633.2): C 47.40, H 5.57, N 6.63; found: C 47.47, H 5.59. N 6.75.

(2,4,6-Tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)-(1 → 3)-1,2,4,6-tetra-*O*-acetyl-D-glucopyranose (20): H_2SO_4 (140 μL of 18 M) was added to a solution of the laminaribioside (**19**) (987 mg, 1.55 mmol), AcOH (7 mL) and Ac_2O (14 mL), and the combined mixtured was stirred (RT, 6 h). NaOAc (1.0 g) was slowly introduced with continued stirring (15 min), and the mixture was filtered. The filtrate was subjected to workup ($CHCl_3$) and flash chromatography (50 – 60% EtOAc/petrol ether) to yield an anomeric mixture of the acetates (**20**) (α/β, 9:1) as a colorless oil (744 mg, 72%); α-anomer: ^{1}H NMR ($CDCl_3$): $\delta = 6.21$ (d, $J(1,2) = 3.7$ Hz, 1H; H-1^{I}), 5.05 – 4.92 (m, 3H; H-2^{I}, H-4^{I,II}), 4.78 (dd, $J(1,2) = 8.0$ Hz, $J(2,3) = 10.1$ Hz, 1H; H-2^{II}), 4.58 (d, $J(1,2) = 8.0$ Hz, 1H; H-1^{II}), 4.32 (dd, $J(5,6) = 4.2$, $J(6,6) = 12.0$ Hz, 1H; H-6^{I}), 4.20 – 4.00 (m, 5H; H-3^{I}, H-5^{I}, H-6^{I,II}), 3.71 – 3.64 (m, 1H; H-5^{II}), 3.51 (t, $J(2,3) = J(3,4) = 10.0$ Hz, 1H; H-3^{II}), 2.15, 2.08, 2.07, 2.06, 2.05, 2.03, 2.01 ppm (7s, 21H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): $\delta =$ 170.63, 170.45, 169.22, 169.20, 168.97, 168.75, 168.62 (7C; C=O), 100.92 (C-1^{II}), 89.11 (C-1^{I}), 76.01 (C-3^{I}), 72.36, 71.23, 70.86, 69.86, 68.18, 67.44 (C-2^{I,II}, C-4^{I,II}, C-5^{I,II}), 64.24 (C-3^{II}), 61.66, 61.65 (C-6^{I,II}), 20.83, 20.63, 20.54, 20.48, 20.33, 20.29 ppm (7C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{26}H_{35}N_3O_{17}$Na: 684.4864; found: 684.1859.

(2,4,6-Tri-*O*-acetyl-3-azido-3-deoxy-β-d-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-acetyl-α-D-glucopyranosyl fluoride (21): HF (4 mL of 70% in pyridine) was added to a cooled (0 °C) polyethylene vessel containing the heptaacetate (**20**) (192 mg, 0.29 mmol), and the mixture was stirred (4 °C; 12 h). CH_2Cl_2 (6 mL) was added, and the combined solution poured onto a stirred suspension of ice in aq. NH_3 (60 mL of 3 M). The solution was subjected to workup (CH_2Cl_2) and flash chromatography (40 – 60% EtOAc/petrol ether) to give the fluoride (**21**) as fine needles (156 mg, 86%); m.p. 175 – 176 °C (EtOH); $[\alpha]_D = +9.9°$ ($c = 0.7$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta =$ 5.63 (dd, $J(1,2) = 2.7$ Hz, J(H,F) 53.2 Hz, 1H; H-1^{I}), 5.03 (t, $J(3,4) = J(4,5) = 9.9$ Hz, 1H; H-4^{II}), 4.93 (t, $J(3,4) = J(4,5) = 9.9$ Hz, 1H; H-4^{I}), 4.89 (ddd, $J(1,2) = 2.7$ Hz, $J(2,3) = 10.2$ Hz, $J(2,F) = 24.7$ Hz, 1H; H-2^{I}), 4.78 (dd, $J(1,2) = 8.0$ Hz, $J(2,3) = 10.2$ Hz, 1H; H-2^{II}), 4.57 (d, $J(1,2) =$ 8.0 Hz, 1H; H-1^{II}), 4.29 (dd, $J(5,6) = 4.7$ Hz, $J(6,6) = 12.6$ Hz, 1H; H-6^{II}), 3.64 (ddd, $J(5,6) = 2.4$ Hz, $J(5,6) = 4.6$ Hz, $J(4,5) = 9.9$ Hz, 1H; H-5^{II}), 3.52 (t, $J(2,3) = J(3,4) = 10.2$ Hz, 1H; H-3^{II}), 2.08, 2.05, 2.04, 2.03, 2.01, 1.99 ppm (6s, 18H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$) $\delta = 170.55$, 170.43, 169.49, 169.03, 168.96, 168.68 (C=O), 103.97 (d, $J(1,F) = 227$ Hz, C-1^{I}), 101.03 (C-1^{II}), 75.59, 72.61, 70.86, 68.14, 66.83 (C-2^{I}, C-4^{I,II}, C-5^{I,II}), 72.18 (d, $J(2,F) =$ 24.1 Hz, C-2^{I}), 70.09 (d, $J(3,F) = 3.7$ Hz, C-3^{I}), 64.33 (C-3^{II}), 61.71, 61.39 (C-6^{I,II}), 20.65, 20.57, 20.52, 20.33 ppm (6C; C=O*C*H$_3$); MS: *m/z*: 644 [M^++Na]; elemental analysis calcd (%) for $C_{24}H_{32}FN_3O_{16}$ (621.2): C 46.39, H 5.19, N 6.76; found: C 46.45, H 5.19, N 6.85.

(3-Azido-3-deoxy-β-D-glucopyranosyl)-(1 → 3)-α-D-glucopyranosyl fluoride (15): NaOMe (100 μL of 1.0 M in MeOH) was added to a solution of the hexaacetate (**21**) (317 mg, 0.51 mmol) in MeOH (10 mL), and the mixture was stirred (0 °C → RT, 2 h). The solution was treated with Amberlite IR-120 resin (H^+ form) until neutral, before filtration. The solvent was evaporated and the residual oil lyophilized (H_2O) to yield the fluoride (**18**) as a white foam (185 mg, 99%); MS: *m/z*: 392 [M^++Na].

***p*-Nitrophenyl (3-azido-3-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-glucopyranosyl)-(1 → 3)-β-D-glucopyranoside (22)**: Condensation of the fluoride (**15**) (1.2 equiv) and *p*-nitrophenyl β-D-glucoside (**2**) (1.0 equiv) yielded the trisaccharide (**22**) as an amorphous powder (81%); ^{1}H NMR (D_2O) $\delta =$ 8.15 – 8.09, 7.13 – 7.09 (2m, 4H; ArH), 5.10 (d, $J(1,2) = 7.5$ Hz, 1H; H-1^{I}), 4.61, 4.59 (2d, $J(1,2) = 8.0$ Hz, $J(1,2) = 7.6$ Hz, 2H; H-1^{II,III}), 3.75 – 3.13 ppm (m, 18H; H-2$^{I–III}$, H-3$^{I–III}$, H-4$^{I–III}$, H-5$^{I–III}$, H-6$^{I–III}$); ^{13}C NMR (D_2O): $\delta =$ 161.95, 143.20 (2C; ArC), 127.53, 118.01 (4C; ArCH), 104.20, 103.92, 100.56 (C-1$^{I–III}$), 85.84, 85.53 (C-3^{I,II}), 78.04, 77.31, 77.07, 74.55, 73.82, 73.57, 69.90, 69.80, 69.51, 69.20 (C-2$^{I–III}$, C-3^{III}, C-4$^{I–III}$, C-5$^{I–III}$), 62.07, 61.91, 61.83 ppm (C-6$^{I–III}$).

A small sample of the trisaccharide was treated with Ac_2O/pyridine/*N*,*N*-dimethylaminopyridine (12 h) and then subjected to workup ($CHCl_3$) and flash chromatography (50 – 70% EtOAc/petrol ether) to yield *p*-nitrophenyl (2,4,6-tri-*O*-acetyl-3-azido-3-deoxy-β-D-glucopyranosyl)-(1 → 3)-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside as a colorless oil; $[\alpha]_D = -43.5°$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.24$ (t, $J(1,2) = J(2,3) = 8.0$ Hz, 1H; H-2^{III}), 5.07 (d, $J(1,2) =$ 7.5 Hz, 1H; H-1^{I}), 4.99-4.87 (m, 4H; H-2^{II}, H-4$^{I–III}$), 4.78 (dd, $J(1,2) =$ 7.8 Hz, $J(2,3) = 10.2$ Hz, 1H; H-2^{I}), 4.49, 4.45 (2 d, $J(1,2) = 8.2$ Hz, $J(1,2) = 8.0$ Hz, 2H; H-1^{II,III}), 4.35 – 4.16, 4.10 – 3.87, 3.82 – 3.57 (3m, 11H; H-3^{II,III}, H-5$^{I–III}$, H-6$^{I–III}$), 3.50 (t, $J(2,3) = J(3,4) = 10.2$ Hz, 1H; H-3^{I}), 2.14, 2.11, 2.10, 2.07, 2.05, 2.04, 2.03, 2.02, 1.99 ppm (9s, 27H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): $\delta = 170.48$, 170.41, 169.11, 169.01, 168.70, 168.67 (9C C=O), 161.17, 143.22 (2C; ArC), 125.73, 116.52 (4C; ArCH), 101.16, 100.59, 97.94 (C-1$^{I–III}$), 78.63, 77.67 (C-3^{I,II}), 72.82, 72.45, 71.87, 70.60, 68.35, 68.20, 68.17 (9C; C-2$^{I–III}$, C-4$^{I–III}$, C-5$^{I–III}$), 64.33 (C-3^{III}), 62.09, 62.03, 61.77 (C-6$^{I–III}$), 20.91, 20.71, 20.66, 20.61, 20.52, 20.45, 20.44 ppm (9C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{42}H_{52}N_4O_{26}$Na: 1051.2767; found: 1051.2775.

***p*-Nitrophenyl (3-azido-3-deoxy-β-D-glucopyranosyl)-(1 → 3)-(β-D-glucopyranosyl)-(1 → 3)-(β-D-glucopyranosyl)-(1 → 3)-β-D-glucopyranoside (23)**: Condensation of the fluoride **15** (10 equiv) and *p*-nitrophenyl β-D-laminaribioside (**14**)[19] (1.0 equiv) yielded the trisaccharide **23** (95%) as a colorless powder; ^{1}H NMR (D_2O): $\delta = 8.24 – 8.18$, 7.22 – 7.17 (2m, 4H; ArH), 5.23 (d, $J(1,2) = 7.7$ Hz, 1H; H-1^{I}), 4.77, 4.76, 4.73 (3d, $J(1,2) =$ 7.9 Hz, $J(1,2) = 8.1$ Hz, 3H; H-1$^{II–IV}$), 3.91 – 3.28 (m, 24H, H-2$^{I–IV}$, H-3$^{I–IV}$, H-4$^{I–IV}$, H-5$^{I–IV}$, H-6$^{I–IV}$); ^{13}C NMR (D_2O): $\delta = 161.67$, 142.67 (2C; ArC), 126.10, 116.52 (4C; ArCH), 102.69, 102.51, 99.25 (4C; C-1$^{I–IV}$), 85.84, 85.53, 84.11 (C-3$^{I–III}$), 76.56, 75.91, 75.62, 73.26, 72.55, 72.15, 68.58, 68.30, 68.09, 68.06, 67.79 (13C; C-2$^{I–IV}$, C-3^{IV}, C-4$^{I–IV}$, C-5$^{I–IV}$), 61.01, 60.67, 60.47, 60.41 ppm (C-6$^{I–IV}$); HRMS-FAB: *m/z* calcd for $C_{30}H_{44}N_4O_{22}$Na 835.2345; found: 835.2339.

Methyl (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-2-*O*-benzyl-4,6-*O*-benzylidine-α-D-glucopyranoside (27): Freshly activated molecular sieves (powdered 4 Å, 500 mg) was added to a solution of α-D-galactosyl trichloroacetimidate (**25**)[24] (1.04 g, 2.1 mmol) and alcohol **26**[25] (604 mg, 1.6 mmol) in CH_2Cl_2 (25 mL), and the combined mixture stirred (RT, 15 min). TMSOTf (30 μL) was added dropwise with continued stirring (10 min), followed by Et_3N (200 μL). The mixture was filtered and evaporated and the residue was subjected to flash chromatography (30 – 35% EtOAc/petrol ether) to yield the title compound (**27**) as powder (968 mg, 85%); ^{1}H NMR ($CDCl_3$): δ7.48 – 7.43, 7.35 – 7.28 (2m, 10H; ArH), 5.29 (dd, $J(3,4) = 3.2$ Hz, $J(4,5) = 0.8$ Hz, 1H; H-4^{II}), 5.26 (dd, $J(1,2) =$ 8.2 Hz, $J(2,3) = 10.6$ Hz, 1H; H-2^{II}), 4.94 (dd, $J(3,4) = 3.4$ Hz, $J(2,3) =$ 10.4 Hz, 1H; H-3^{II}), 4.80 (d, $J(1,2) = 8.2$ Hz, 1H; H-1^{II}), 4.76, 4.48 (2d, J(H,H) = 12.0 Hz, 2H; C*H*$_2$Ph), 4.42 (d, $J(1,2) = 3.8$ Hz, 1H; H-1^{I}), 4.22 – 4.01 (m, 3H; H-4^{II}, H-6^{I,II}), 3.83 – 3.564 (m, 6H; H-3^{I}, H-5^{I,II}, H-6^{I,II}, PhC*H*), 3.50 (dd, $J(1,2) = 3.8$ Hz, $J(2,3) = 9.3$ Hz, 1H; H-2^{I}), 3.32 (s, 3H; OMe), 2.10, 1.97, 1.94, 1.89 ppm (4s, 12H; C=OCH$_3$); ^{13}C NMR ($CDCl_3$): $\delta =$ 170.21, 170.13, 170.19, 169.55 (4C; C=O), 138.09, 137.24, 129.08, 128.59, 128.48, 128.24, 128.09, 128.00, 127.85, 125.97 (12C; Ar), 101.44, 101.33 (C-1^{II}, PhC*H*), 98.97 (C-1^{I}), 80.78, 78.66, 78.60, 74.10, 71.20, 70.63, 69.62, 68.99, 67.00 (C-2^{I,II}, C-3^{I,II}, C-4^{I,II}, C-5^{I,II}, *C*H$_2$Ph), 61.95, 60.89 (C-6^{I,II}), 55.33 (OMe), 20.79, 20.61, 20.52, 20.50 ppm (4C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{35}H_{42}O_{15}$Na: 725.2421; found: 725.2429.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 3)-1,2,4,6-tetra-*O*-acetyl-D-glucopyranose (28): Palladium on charcoal (55 mg of 10%) was added to a solution of the tetraacetate (**27**) (550 mg, 0.78 mmol) and glacial acetic acid (50 μL) in a mixture of MeOH (10 mL) and EtOAc (5 mL), and the mixture vigorously stirred under an atmosphere of hydrogen (6 – 12 h). The mixture was filtered, evaporated, and treated with H_2SO_4 (200 μL of 18 M), AcOH (5 mL), and Ac_2O (10 mL), and the combined mixtured stirred (RT, 12 h). NaOAc (1.0 g) was introduced slowly with continued stirring (15 min) and the mixture was filtered. The filtrate was subjected to workup ($CHCl_3$) and flash chromatography (60 – 70% EtOAc/petrol ether) to yield an anomeric mixture of the acetate (**28**) (α/β, 9:1) as a colourless oil (488 mg, 92%); $[\alpha]_D = +32.0°$ ($c = 1.0$ in $CHCl_3$; lit[30] $+29.0°$); α-anomer: ^{13}C NMR ($CDCl_3$) $\delta = 170.67$, 170.39, 170.18, 170.10, 169.32, 169.00, 168.63 (8C; C=O), 101.05 (C-1^{II}), 89.17 (C-1^{I}), 75.64 (C-3^{I}), 71.23, 70.89, 70.39, 69.92, 69.88, 67.62, 66.68 (C-2^{I,II}, C-3^{II}, C-4^{I,II}, C-5^{I,II}), 61.73, 60.69 (C-6^{I,II}), 20.90, 20.68, 20.59, 20.56, 20.48, 20.39 ppm (8C; C=O*C*H$_3$); HRMS-FAB: *m/z* calcd for $C_{28}H_{38}O_{19}$Na: 701.1905; found: 701.1905.

(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-α-D-glucopyranosyl fluoride (29): HF (6 mL of 70% in pyridine) was added to a cooled (0°C) polyethylene vessel containing the heptaacetate (**28**) (247 mg, 0.36 mmol), and the mixture was stirred (4°C; 12 h). CH_2Cl_2 (6 mL) was added and the combined solution poured onto a stirred suspension of ice in aq. NH_3 (60 mL of 3M). The solution was subjected to workup (CH_2Cl_2) and flash chromatography (40–60% EtOAc/petrol ether) to give the fluoride **29** as a colorless oil (168 mg, 72%). ^{1}H NMR ($CDCl_3$): $\delta = 5.63$ (dd, $J(1,2) = 2.7$ Hz, $J(1,F) = 53.2$ Hz, 1H; H-1^I), 5.34–5.32 (m, 1H; H-4^{II}), 5.09–5.01, 4.96–4.82 (2m, 4H; H-2^{I,II}, H-3^{II}, H-4^I), 4.59 (d, $J(1,2) = 7.9$ Hz, 1H; H-1^{II}), 4.22–4.00 (m, 7H; H-3^I, H-5^{I,II}, H-6^{I,II}), 2.14, 2.10, 2.06, 2.03, 2.02, 1.94, 1.93 ppm (7s, 21H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.53$, 170.26, 170.12, 170.06, 169.59, 169.01, 168.93 (7C; C=O), 103.98 (d, $J(1,F) = 227$ Hz, C-1^I), 101.07 (C-1^{II}), 75.17 (C-3^I), 72.10 (d, $J(2,F) = 24.1$ Hz, C-2^I), 70.95, 70.59, 68.91, 67.05, 66.83 (C-2^{II}, C-4^{I,II}, C-5^{II}), 70.09 (d, $J(3,F) = 3.7$ Hz, C-3^I), 61.44, 60.92 (C-6^{I,II}), 20.65, 20.52, 20.45, 20.36 ppm (7C; C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{26}H_{35}FO_{17}Na$: 661.1756; found: 661.1759.

(β-D-Galactopyranosyl)-(1→3)-α-D-glucopyranosyl fluoride (24): NaOMe (50 μL of 1.0M in MeOH) was added to a solution of the heptaacetate (**29**) (85 mg, 0.13 mmol) in MeOH (5 mL), and the mixture stirred (0°C→RT, 2 h). The solution was treated with Amberlite IR-120 resin (H^+ form) until neutral, filtered, the solvent evaporated and the residual oil lyophilized (H_2O) to yield the fluoride (**24**) as a white foam (45 mg, 99%); MS-FAB: *m/z*: 367 [M^++Na].

***p*-Nitrophenyl (2,3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-(1→3)-(2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (30)**: The fluoride **24** and *p*-nitrophenyl β-D-glucoside (**2**) were subjected to the glycosynthase reaction (12 h), and the solution was lyophilized. The residue was treated with Ac_2O (2 mL), pyridine (3 mL), and *N*,*N*-dimethylaminopyridine (100 mg), and the combined solution stirred (RT, 12 h). The mixture was subjected to workup ($CHCl_3$) and flash chromatography to yield the trisaccharide **30** as a colorless oil (65%); $[\alpha]_D = -17.9°$ ($c = 1.1$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.20–8.14$, 7.08–6.98 (2m, 4H; Ar), 5.33 (dd, $J(3,4) = 3.3$ Hz, $J(4,5) = 0.8$ Hz, 1H; H-4^{III}), 5.25 (dd, $J(1,2) = 7.7$, $J(2,3) = 8.6$ Hz, 1H; H-2^I), 5.07 (d, $J(1,2) = 7.9$ Hz, 1H; H-1^I), 5.02 (t, $J(3,4) = J(4,5) = 7.8$ Hz, 1H; H-4^{II}), 4.97–4.90 (m, 3H; H-2^{II,III}, H-4^I), 4.90 (dd, $J(3,4) = 3.5$ Hz, $J(2,3) = 9.1$ Hz, 1H; H-3^{III}), 4.49, 4.46 (2d, $J(1,2) = 7.7$ Hz, $J(1,2) = 7.8$ Hz, 2H; H-1^{II,III}), 4.30 (dd, $J(5,6) = 4.4$ Hz, $J(6,6) = 12.2$ Hz, 1H; H-6^{II}), 4.23–4.16, 4.09–3.80 (2m, 9H; H-3^{I,III}, H-5^{II,III}, H-6$^{I–III}$), 3.70–3.63 (m, 1H; H-5^I), 2.16, 2.12, 2.09, 2.06, 2.04, 2.03, 2.02, 2.00, 1.94 ppm (10s, 30H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.09–168.71$ (10C; C=O), 161.22, 143.26 (2C; ArC), 125.76, 116.55 (4C; ArCH), 101.25,100.77, 98.03 (C-1$^{I–III}$), 78.38, 77.77 (C-3^{I,II}), 72.69, 72.48, 72.41, 71.88, 70.98, 70.32, 68.51, 68.40, 68.18, 66.63 (C-2$^{I–III}$, C-3^{III}, C-4$^{I–III}$, C-5$^{I–III}$), 62.14, 62.05, 60.64 (C-6$^{I–III}$), 20.96, 20.67, 20.57, 20.47 ppm (10C; C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{44}H_{55}NO_{28}Na$: 1068.2808; found: 1068.2780.

(β-D-Glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→3)-α-D-glucopyranosyl fluoride (31): i) HF (4 mL of 70% in pyridine) was added to a cooled (0°C) polyethylene vessel containing (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-D-glucopyranose[27] (210 mg, 0.21 mmol), and the mixture was stirred (4°C; 12 h). CH_2Cl_2 (6 mL) was added, and the combined solution poured onto a stirred suspension of ice in aq. NH_3 (60 mL of 3M). The solution was subjected to workup (CH_2Cl_2) and flash chromatography (60% EtOAc/petrol ether) to give the (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→3)-1,2,4,6-tetra-*O*-acetyl-D-glucopyranosyl fluoride as a white foam (145 mg, 72%); $[\alpha]_D = +1.1°$ ($c = 0.3$ in $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 5.62$ (dd, $J(1,2) = 2.7$ Hz, $J(1,F) = 53.4$ Hz, 1H; H-1^I), 5.11 (t, $J(2,3) = J(3,4) = 9.6$ Hz, 1H; H-3^{III}), 5.09 (t, $J(3,4) = J(4,5) = 9.5$ Hz, 1H; H-4^I), 5.03 (dd, $J(3,4) = 9.6$ Hz, $J(2,3) = 10.0$ Hz, 1H; H-3^I), 5.02 (t, $J(3,4) = J(4,5) = 9.5$ Hz, 1H; H-4^{III}), 4.91 (dd, $J(1,2) = 7.9$ Hz, $J(2,3) = 9.0$ Hz, 1H; H-2^{III}), 4.90 (ddd, $J(1,2) = 2.6$ Hz, $J(2,3) = 10.0$, $J(2,F) = 25.0$ Hz, 1H; H-2^I), 4.81 (dd, $J(1,2) = 8.2$ Hz, $J(2,3) = 9.7$ Hz, 1H; H-2^{II}), 4.61 (d, $J(1,2) = 8.2$ Hz, 1H; H-1^{III}), 4.46 (d, $J(1,2) = 7.9$ Hz, 1H, H-1^{II}), 4.43–4.01 (m, 8H; H-3^I, H-5^{II}, H-6$^{I–III}$), 3.75 (t, $J(3,4) = J(4,5) = 9.0$ Hz, 1H; H-4^{II}), 3.65–3.55 (m, 2H; H-5^{I,III}), 2.17, 2.10, 2.07, 2.06, 2.01, 1.99, 1.98, 1.97, 1.95, 1.94 ppm (10s, 30H; C=OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.59$, 170.44, 170.17, 170.09, 169.84, 169.76, 169.29, 169.02, 168.99 (10C; C=O), 103.81 (d, $J(1,F) = 227$ Hz, C-1^I), 100.79 (2C; C-1^{II,III}), 77.19, 76.21, 75.76, 72.87, 72.79, 72.07, 71.62, 71.39, 67.82, 66.86 (C-2^{II,III}, C-3$^{I–III}$, C-4$^{I–III}$, C-5^{II,III}), 72.55 (d, $J(2,F) = 24.0$ Hz; C-2^I), 70.09 (d, $J(3,F) = 3.7$ Hz, C-5^I), 62.04, 61.57, 61.40 (C-6$^{I–III}$), 20.74, 20.69, 20.64, 20.49, 20.47, 20.37, 20.32 ppm (10C; C=OCH_3); HRMS-FAB: *m/z* calcd for $C_{38}H_{51}FO_{25}Na$ 949.2601; found: 949.2586.

ii) NaOMe (50 μL of 1.0M in MeOH) was added to a solution of the previously described decaacetate synthesized above (87 mg, 94 μmol) in MeOH (5 mL) and the mixture stirred (0°C→RT, 2 h). The solution was treated with Amberlite IR-120 resin (H^+ form) until neutral, filtered, the solvent evaporated and the residue lyophilised (H_2O) to yield the title fluoride (**31**) as a white foam (47 mg, 99%); MS: *m/z*: 529 [M^++Na].

***p*-Nitrophenyl (β-D-glucopyranosyl)-(1→4)-(β-D-glucopyranosyl)-(1→3)-(β-D-glucopyranosyl)-(1→3)-β-D-glucopyranoside (32)**: Condensation of fluoride **31** and *p*-nitrophenyl β-D-glucoside (**2**) yielded the tetrasaccharide **32** as an amorphous powder (33%); ^{1}H NMR (D_2O): $\delta = 8.25–8.19$, 7.23–7.16 (2m, 4H; ArH), 5.24 (d, $J(1,2) = 7.3$ Hz, 1H; H-1^I), 4.77, 4.73 (2d, $J(1,2) = 8.0$ Hz, 2H; H-1^{II,III}), 4.44 (d, $J(1,2) = 7.7$ Hz, 1H; H-1^{IV}), 3.94–3.22 (m, 24H; H-2$^{I–IV}$, H-3$^{I–IV}$, H-4$^{I–IV}$, H-5$^{I–IV}$, H-6$^{I–IV}$); ^{13}C NMR (D_2O): $\delta = 163.10$, 145.23 (2C; ArC), 127.53, 117.95 (4C; ArCH), 103.96, 100.68 (4C; C-1$^{I–IV}$), 85.52, 85.14 (C-3^{I,II}), 80.06 (C-4^{III}), 77.34, 77.04, 76.91, 76.24, 75.55, 74.66, 74.57, 73.98, 70.88, 69.50, 69.2 (13C; C-2$^{I–III}$, C-3^{III,IV}, C-4^{I,III,IV}, C-5$^{I–III}$), 61.07, 60.79 ppm (3C; C-6$^{I–III}$); HRMS-FAB: *m/z* calcd for $C_{30}H_{45}NO_{23}Na$ 810.2280; found: 810.2266.

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