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New oligo-β-(1,3)-glucan derivatives as immunostimulating agents

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ABSTRACT

Oligo-β-(1,3)-glucans were chemically modified in order to introduce a structural variation specifically on the reducing end of the oligomers. The impact of well defined structural modulations was further studied on cancer cells and murin models to evaluate their cytotoxicity and immunostimulating potential.

1. Introduction

It is now well established that natural β-(1,3)-glucans, which are neither biosynthesized nor metabolized by mammals, are potent immunological activators. Recent studies give evidence for renewed interest in these polysaccharides in the health domain,[1–3] particularly with those related to clinical trials where they are used as bioactive adjuvants in the treatment of patients receiving chemotherapies.[4,5] These studies notably highlight improvement of the quality of life of cancer patients—a very important criterion in oriental countries. During the last decade, the main receptors able to interact specifically with β-(1,3)-glucans were identified. Amongst them are the dectin-1,[6,7] the complement receptor of type 3 (CR3),[8–10] a scavenger and a lactosylceramide. Since these receptors are widely distributed on various immunocompetent cells, β-(1,3)-glucans impact on both the innate and the adaptive immunity. The related biological effects were recently reviewed favorably.[1,3] It was also shown that therapies based on antitumor monoclonal antibodies are enhanced by administered β-glucans.[11–13] However, the structure–function relationships are still objects of debate.[2,14,15] It results from biological data obtained until now from polysaccharides that poor correlations can be established with structural and conformational requirements, molecular weight and degree of branching. In summary, glucans have been extensively studied for their immunological and pharmacological effects. More than 10,000 papers describing the biological activities of glucans exist.[16] However, many of the available data were obtained from extracted materials. As a result, neither chemical structure nor purity of actual bioactive molecule(s) can be rigorously specified. The absence of direct benefits of polysaccharides on health therefore dampens the development in western countries of such complementary and alternative medicine. It is important, therefore, to evaluate the possibility of using synthetic oligosaccharides based on the structure of glucans.

In this context, and in order to increase our knowledge of laminarine extracted from brown seaweeds,[17] we have recently initiated a program devoted to the chemical synthesis of well defined and pure oligo-β-(1,3)-glucans.[18] As a result, while they are not able to adopt any helical arrangements, small linear oligo-β-(1,3)-glucans possess biological activity comparable to that of the native Phycarine®. Consequently, compared with polysaccharides, minor chemical variations on small oligoglucans may present significant changes in interactions with the targeted receptors. These effects are expected to give a great deal of information on the respective importance of hydroxyl groups. Upon this basis, we now present a first chemical modulation that results in a new family of oligo-β-(1,3)-glucans (Fig. 1) modified on the reducing end. The designed glycosides are characterized by the presence of four or five glucopyranose entities and a mannose residue at the reduc-

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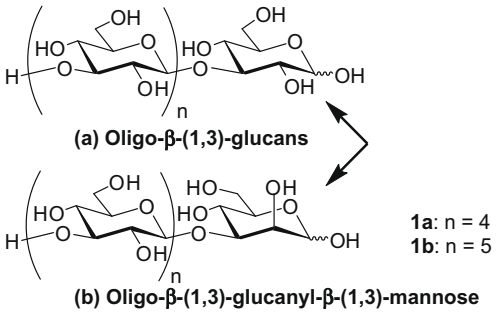


Figure 1. Structure of oligo-β-(1,3)-glucan-mannose conjugates.

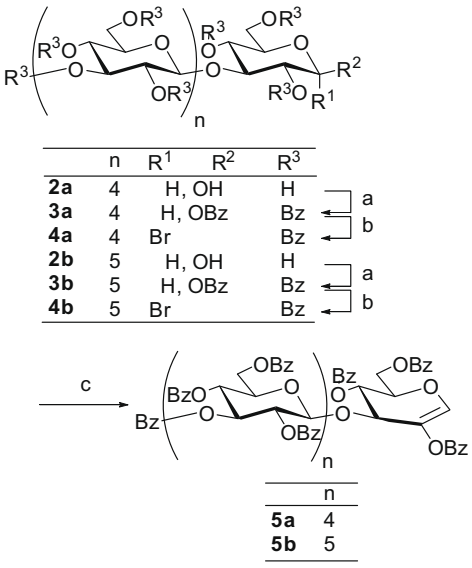
ing end. The impact of fine chemical variation on the phagocytic activity and the secretion of interleukins was also studied in a murine model.

2. Results and discussion

2.1. Synthesis of oligo-β-(1,3)-glucanyl-β-(1,3)-mannose

The introduction of a mannose entity on an oligoglucan chain could be based on three different approaches: (1) a total synthesis of the oligosaccharidic chain thanks to standard tools of glycochemistry, (2) a coupling between an oligomer as donor and a suitably protected mannopyranosyl acceptor, and (3) a controlled and specific inversion of configuration at C-2 starting from already formed oligo-β-(1,3)-glucans. In order to optimize time dedicated to synthetic work, and because it is well known that reactivity of glycosyl donors decreases by increasing the number of glycosyl residues, we expected that the latter strategy would be the most appropriate. This purpose required to distinguish the OH-2 group on the reducing end from all the other OH-2 functions. Such an approach, first introduced by Lichtenthaler for the preparation of β-mannopyranosides, involves the reduction of a 2-keto-glycoside which is obtained by glycosidation of an ulosyl bromide, resulting from bromination of a glucan derivative.[19,20] Full experimental details and mechanistic analysis were described, included those related to the formation of by-products.[21] Still limited to the modification of mono- and disaccharides, we attempted to extend this concept to laminaripenta- and hexaoses, **2a** and **2b**, respectively (Scheme 1). Therefore, perbenzoylation of **2a** and **2b**, followed by anomeric bromination of the reducing end, were performed under standard conditions to afford glucanyl bromides **4a** and **4b**, respectively. The synthesis of glucans **5a** and **5b** required an elimination reaction with the assistance of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Both enol ethers were thus isolated in yields upper to 80%.

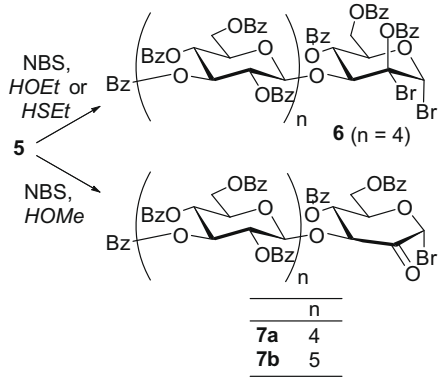
An additional step consisted of electrophilic bromination of the ethylenic bond using *N*-bromosuccinimide (NBS) in situ followed by oxidation of the C-2 position thanks to the action of ethanol on the 2-benzoic ester (Scheme 2). However, under these initial conditions, the non-expected dibromide derivative **6**[21] was obtained from the pentasaccharidic glycal in a low yield. At this stage, we supposed competing reactivity between bromide ion, which adds to bromonium intermediate, and ethanol, which acts on carbonyl group of the C-2 ester group to induce the formation of the keto function. To corroborate this hypothesis, ethanol was first substituted with ethanethiol in order to induce in situ production of bromine thanks to an oxido-reduction reaction. As a result, **6** was obtained quantitatively. Its structure was established on the basis of a new signal on the ^{13}C NMR spectrum at 89.9 ppm corresponding to C-2a, and on conformational changes, from a half-chair conformation ^4H for **5a** to a boat $^4C_1$ for **6**. The value of the coupling



	n	R^1	R^2	R^3	
2a	4	H, OH		H	a
3a	4	H, OBz		Bz	b
4a	4	Br		Bz	
2b	5	H, OH		H	a
3b	5	H, OBz		Bz	b
4b	5	Br		Bz	

	n
5a	4
5b	5

Scheme 1. Synthesis of glycols **5**. Reagents: (a) BzCl, pyridine (**3a**: 94%; **3b**: 88%); (b) HBr, HOAc (**4a**: 100%; **4b**: 88%); (c) DBU (**5a**: 81%; **5b**: 85%).

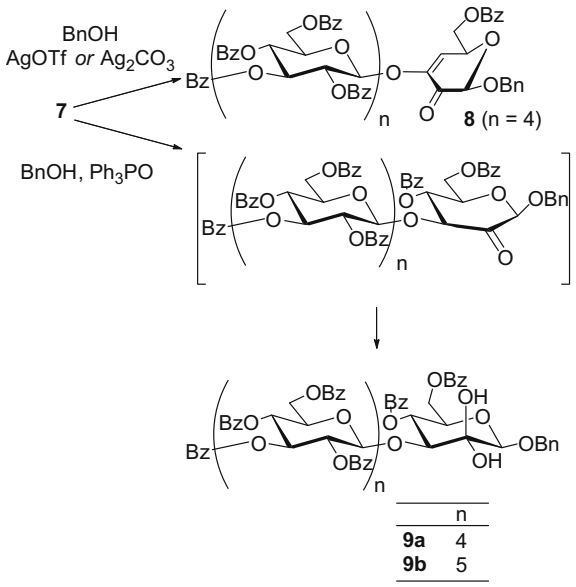


	n
7a	4
7b	5

Scheme 2. Synthesis of ulosyl bromides **7**. Reagents: NBS, HSEt (from **5a**, $n = 4$, 100%); NBS, HOMe (**7a**: 100%; **7b**: 100%).

constant between H-3a and H-4a of 9.3 Hz, as well as no possible correlation with H-1a, corroborated the ^{13}C NMR data. Subsequently, the same reaction was further performed in the presence of anhydrous methanol. The desired ulosyl bromides **7a** and **7b** were thus obtained in near quantitative yields.

With the required donors in hands, the protection of the anomeric center of the terminal reducing entity was studied under various conditions (Scheme 3). In the presence of benzyl alcohol and silver triflate as a Lewis acidic promoter, the desired glycosylation efficiently occurred but accompanied by an elimination reaction at C-3, C-4. This reaction allowed us to isolate the enone **8** in an excellent 75% yield. A similar result was observed under basic conditions in the presence of silver carbonate. In order to limit the elimination process, the introduction of benzyl alcohol was further attempted under neutral conditions using triphenylphosphine oxide as a promoter.[22] Provided that reactions were carefully controlled, the targeted 2-keto glycosides were thus obtained and isolated in 87% and 85% in the corresponding hydrated form **9a** and **9b**, respectively. The coupling constant between C-1a and H-1a of 171 Hz shows that the β-anomers were specifically synthesized. This was quite surprising since initial studies based on this concept favoured retention of C-1 configuration thanks to a double inversion—the first one by triphenylphosphine oxide on starting bro-



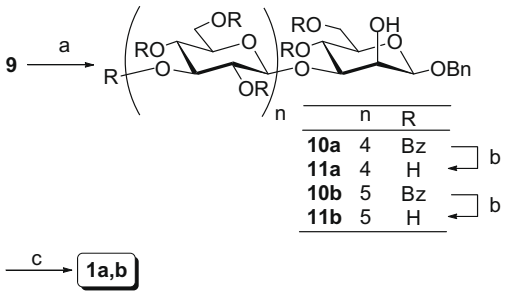
Scheme 3. Glycosylation of benzyl alcohol from ulosyl bromides. Reagents: BnOH, AgOTf (starting from **7a**, $n = 4$, 75%); BnOH, Ph_3PO (**9a**: 87%; **9b**: 85%).

mide and the second one by the acceptor on the intermediate phosphonium. A possible explanation to justify the observed opposite selectivity could be connected with the use of 2 equiv of the neutral promoter. This possibly resulted in a shift of the α,β-equilibrium for the 2-keto phosphonium intermediate towards the α-form, and hence to the synthesis of the β-glycoside.

Further reduction of **9a** and **9b** was efficiently performed with L-Selectride and afforded derivatives **10a** and **10b**, respectively, in near quantitative yields and with total *manno* selectivity (Scheme 4). The target oligo-β-(1,3)-glucanyl-β-(1,3)-mannose were finally obtained after Zemplén transacylation and palladium-catalyzed hydrogenolysis in high degree of purity.

2.2. Immunostimulating effects of glucan-mannose conjugates 1a and 1b

We first verified that our set of new molecules did not induce potential aspecific toxicity onto cultured cells. Assays were performed by monitoring cell viability and growth activity of five different tumor cell lines representative of the most frequent solid tumors in human (liver, colon, prostate, breast, and lung) and one resting normal diploid skin fibroblastic cell line, exposed to increasing concentrations of the molecules (from 0.1 to 25 μM) for 1 and 2 days. None of the tested molecules were able to induce changes in the growth kinetics of actively dividing cells as well as



Scheme 4. Synthesis of mannose derivatives and final deprotection steps. Reagents: (a) L-Selectride (**10a**: 100%; **10b**: 98%); (b) NaOMe, HOMe (**11a**: 91%; **11b**: 96%); (c) H_2, $Pd(OAc)_2$ (**1a**: 100%; **1b**: 100%).

diploid control fibroblastic cells which weakly divided. This confirmed no aspecific toxicity induced by the molecules.

Then, the specific effects of the molecules onto cells involved into the immune response were monitored. The effects of various glucans on macrophages are well established (for a review, see Novak and Větvička[16]). However, in order to demonstrate that synthetic oligosaccharides built up with linear short chains of glucans unable to adopt helical arrangements, really exhibit immunomodulatory characteristics, an evaluation of some of the immune reactions is necessary. Moreover, the effects observed with glucan-mannose conjugates **1a, b** were also compared with those induced by pure laminaripentaose and laminariheptaose (Fig. 1a, $n = 4$ and 6, respectively). We first showed that intra-peritoneal injection of individual samples resulted in an increase in the percentage of granulocytes in peripheral blood. This was accompanied by a decrease of percentage of lymphocytes (Fig. 2). Similarly, all four samples caused an influx of peritoneal macrophages (Fig. 3).

Glucans are well known to stimulate phagocytosis.[23,24] Therefore, in the next step we measured the effects of oligosaccharides on phagocytosis of synthetic HEMA microspheres in peripheral blood (Fig. 4) and peritoneal cavity (Fig. 5). Our results showed that all tested samples significantly increased phagocytic activity of

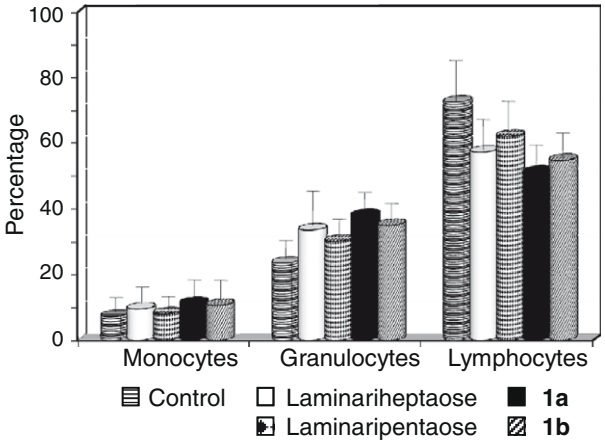


Figure 2. Effect of intra-peritoneal injection of 100 μg of samples on percentages of peripheral blood cells. The results represent the mean of three independent experiments ± SD.

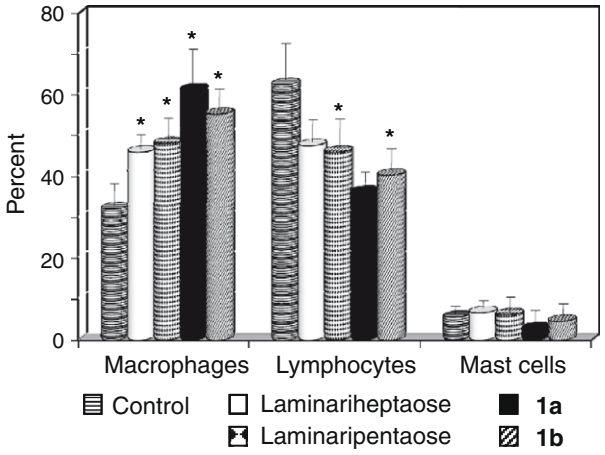


Figure 3. Effect of intra-peritoneal injection of 100 μg of samples on percentages of peritoneal cells. The results represent the mean of three independent experiments ± SD. *represents significant differences between samples and control at $P < 0.05$ level.

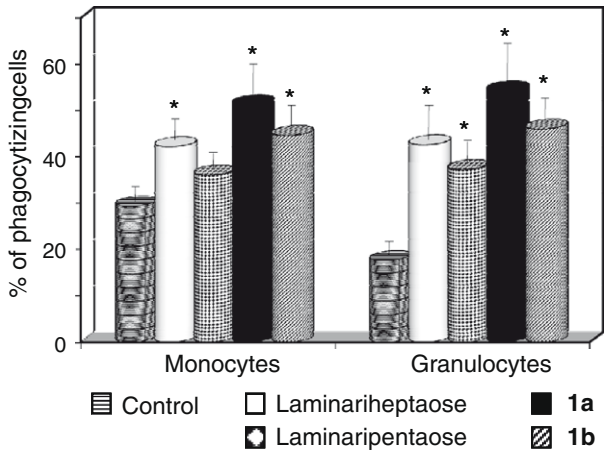


Figure 4. Potentiation of phagocytosis of synthetic microspheres (HEMA particles) by different oligosaccharides injected intra-peritoneally 24 h before test. Monocytes and granulocytes with three and more HEMA particles were considered positive. Each value represents the mean ± SD. All differences were significant at P <0.05 level except when marked as *.

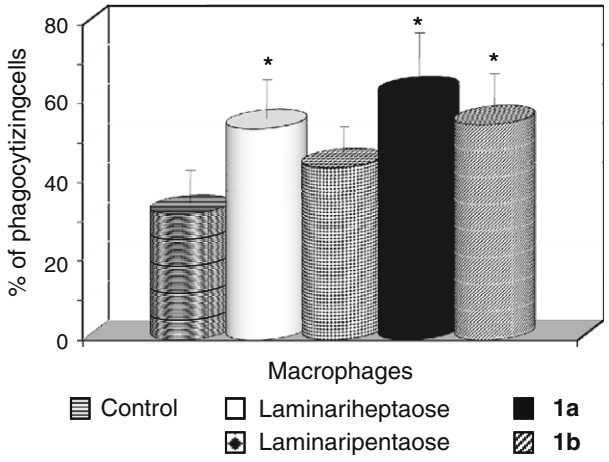


Figure 5. Potentiation of phagocytosis of synthetic microspheres (HEMA particles) by different oligosaccharides injected intra-peritoneal 24 h before test. Peritoneal macrophages with three and more HEMA particles were considered positive. Each value represents the mean ± SD. All differences were significant at P <0.05 level except when marked as *.

both peripheral blood monocytes and granulocytes and peritoneal macrophages. In every case, the highest activity was found with **1a**.

It is hypothesized that the immunostimulating actions of oligo-β-glucans are, at least in part, caused by potentiation of a synthesis and release of several cytokines.[25] To see if our samples influenced the cytokine production, we measured the immunomodulating activity through effects on the production of IL-2 by spleen cells. The production of IL-2 was measured after a 72 h in vitro incubation of spleen cells isolated from control and oligosaccharide-treated mice (Fig. 6). Again, **1a** was the most active sample, able to stimulate IL-2 production to the same level as Concanavalin A (data not shown).

3. Conclusion

The synthesis of small linear neo-oligo-β-(1,3)-glucans was performed in order to evaluate in vivo the impact of structural modifications at the reducing moiety on the activity of macrophages. Our approach relied on controlled inversion of the C-2a configuration starting from an oligosaccharidic chain. This fine structural

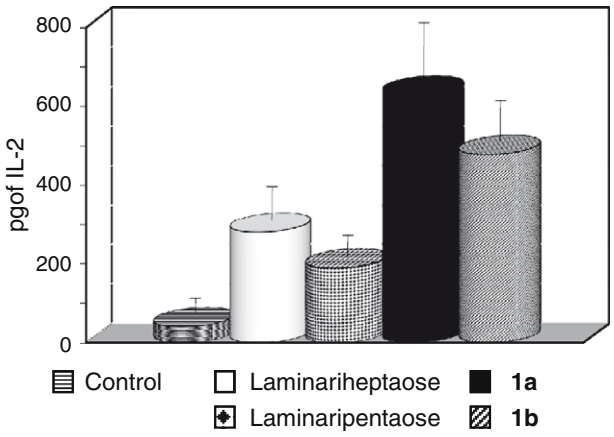


Figure 6. Effect of intra-peritoneal injection of 100 μg of samples on in vitro secretion of IL-2 by spleen cells. Control cells were isolated from mice injected with PBS. The results represent the mean of three independent experiments ± SD. *represents significant differences between samples and control at P <0.05 level.

modulation involved ulosyl bromides as keys intermediates. Introduction of benzyl alcohol on the reducing anomeric center required neutral but neither acidic nor basic activation, otherwise elimination occurred. Further selective reduction by L-Selectride followed by standard deprotection steps gave the desired glucan-mannose conjugates in 60.3% and 52.6% overall yields for eight steps for **1a** and **1b**, respectively. Although they are not able to form helices, these oligosaccharides were however efficient to stimulate immune responses in vivo after intra-peritoneal injection in murin models. Increased ratio of granulocytes in peripheral blood, potentiation of phagocytosis and production of IL-2 represent evidences for the expected immunostimulating effects. It was also interesting to note that impact on the production of interleukin-2 was significantly improved using synthetic glucan-mannose conjugates, and more especially the pentasaccharide **1a**, compared to the effects observed with linear laminarihepta- and pentaose. These results also confirm the biological impact of linear β-(1,3)-glucans unable to form helical structures. Further efforts to strengthen the importance of the reducing end of oligo-β-(1,3)-glucans are in due course.

4. Experimental

4.1. Material and methods

Reactions and chromatographic purifications were monitored by thin layer chromatography (TLC) analyses conducted on Merck 60 F_{254} Silica Gel non-activated plates and compounds were revealed using a 5% solution of H_2SO_4 in EtOH followed by heating. For column chromatography, Geduran Si 60 (40–63 μm) Silica Gel was used. ^1H, ^{13}C, HMQC and COSY NMR spectra were recorded on a Bruker ARX 400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are given in δ-units (ppm) measured downfield from Me_4Si. Optical rotations were measured on a Perkin–Elmer 341 polarimeter. The HRMS were measured at the Centre Régional de Mesures Physiques de l'Ouest (University of Rennes 1, France) with a MS/MS ZabSpec TOF Micromass using *m*-nitrobenzylic alcohol as a matrix and accelerated cesium ions for ionization. Elemental analysis was recorded at the CRMPO on a Flash EA1112 CHN/O microanalyser.

4.2. Benzoylation of oligo-β-(1,3)-glucans

To a solution of unprotected glucan in pyridine cooled at 0 °C was added benzoyl chloride. After stirring for 2 days at room tem-

perature, the reaction media was concentrated, the crude oil diluted in CH_2Cl_2 and the resulting organic layers successively washed with 10% HCl, a saturated aqueous solution of $NaHCO_3$ and brine. It was further dried ($MgSO_4$) and concentrated. A final chromatographic purification gave the desired perbenzoylated oligo-β-(1,3)-glucan as an inseparable mixture of anomers.

4.2.1. Perbenzoylated laminaripentaose (3a)

Compound **3a** was obtained according to the general procedure starting from laminaripentaose (150 mg, 0.181 mmol), pyridine (15 mL), and benzoyl chloride (4.2 mL, 36 mmol). Chromatography eluting with petroleum ether/EtOAc (3:2) gave **3a** (444 mg, 0.170 mmol) in 94% yield.

R_f = 0.4 (1:1 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data for the α-anomer: δ 6.65 (d, 1H, $J_{1a,2a}$ = 3.6 Hz, H-1a), 5.47 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 9.4 Hz, H-4a), 4.64 (t, 1H, $J_{2a,3a}$ = 9.4 Hz, H-3a); significant data for the β-anomer: δ 6.07 (d, 1H, $J_{1a,2a}$ = 7.1 Hz, H-1a), 5.57 (t, 1H, $J_{3a,4a} = J_{H4a,H5a}$ = 8.9 Hz, H-4a), 4.25 (dt, 1H, $J_{5a,6a}$ = 4.3 Hz, $J_{5a-6'a}$ = 8.9 Hz, H-5a); other signals: δ 8.08–7.92 (m, 68H, H arom), 7.61–7.09 (m, 102H, H arom), 5.45–5.37 (m, 3H, H-2aβ, H-3e), 5.26–5.18 (m, 5H, H-2aα, H-2e, H-4e), 5.11–4.83 (m, 14H, H-1b, H-2b, H-4b, H-2c, H-4c, H-2d, H-4d), 4.61–4.35 (m, 10H, H-3aβ, H-5aα, H-6aβ, H-6′aβ, H-1c, H-1d, H-1e), 4.20–3.86 (m, 26H, H-6aα, H-6′aα, H-3b, H-6b, H-6′b, H-3c, H-6c, H-6′c, H-3d, H-6d, H-6′d, H-5e, H-6e, H-6′e), 3.80–3.65 (m, 6H, H-5b, H-5c, H-5d); ^{13}C NMR ($CDCl_3$): significant data for the α-anomer: δ 87.7 (C-1a), 76.3 (C-3a), 72.4 (C-2a), 70.3 (C-5a), 67.6 (C-4a); significant data for the β-anomer: δ 89.7 (C-1a), 78.4 (C-3a), 72.4 (C-2a, C-5a), 67.6 (C-4a), 62.0 (C-6a); other chemical shifts: 166.0, 165.9, 165.8, 165.7, 165.6, 165.4, 164.9, 164.8, 164.7, 164.6, 164.5, 164.3, 164.1, 163.8, 163.6, 163.5 (OCOPh), 133.3, 132.7, 130.0, 129.3, 129.2, 129.1, 129.0, 128.9, 128.7 (C_{quat} arom), 133.8, 133.6, 133.4, 133.2, 133.1, 133.0, 132.9, 132.8, 132.6, 129.9, 129.7, 129.6, 129.5, 129.4, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9 (C arom), 101.4, 101.3, 101.0, 100.8, 100.7 (C-1b, C-1c, C-1d, C-1e), 78.1, 77.5, 77.4, 77.2 (C-3b, C-3c, C-3d), 73.4, 73.3, 73.2 (C-2), 72.7, 72.6, 72.5, 72.4 (C-3e, C-2), 71.8, 71.7, 71.6, 71.5 (C-5b, C-5c, C-5d, C-5e), 71.3 (C-2e), 70.3, 70.2 (C-4b, C-4c, C-4d), 69.8 (C-4e), 63.5, 63.4, 63.1, 62.7 (C-6a, C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2619.7110 $[M+Na]^+$, calcd for $C_{149}H_{120}NaO_{43}$ 2619.7101.

4.2.2. Perbenzoylated laminarihexaose (3b)

The title compound was obtained according to the general procedure starting from laminarihexaose (200 mg, 0.202 mmol), pyridine (20 mL), and benzoyl chloride (5.2 mL, 44.4 mmol). Chromatography eluting with petroleum ether/EtOAc (1:1) gave **3b** (545 mg, 0.178 mmol) as a colorless oil in 85% yield.

R_f = 0.4 (1:1 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data for the α-anomer: δ 6.60 (d, 1H, $J_{1a,2a}$ = 3.8 Hz, H-1a), 5.42 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 9.8 Hz, H-4a); selected chemical shifts for the β-anomer: δ 6.02 (d, 1H, $J_{1a,2a}$ = 7.1 Hz, H-1a), 5.52 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 9.0 Hz, H-4a), 4.23–4.18 (m, 1H, H-5a); other signals: δ 8.01–7.65 (m, 60H, H arom), 7.55–7.01 (m, 140H, H arom), 5.36–5.30 (m, 3H, H-2aβ, H-3f), 5.20–5.12 (m, 5H, H-2aα, H-2f, H-4f), 5.07–4.89 (m, 8H, 2H-2, 2H-4), 4.88–4.72 (m, 10H, H-1b, 2H-2, 2H-4), 4.61–4.30 (m, 13H, H-3aα, H-3aβ, H-5aα, H-6aβ, H-6′aβ, H-1c, H-1d, H-1e, H-1f), 4.12–3.78 (m, 32H, H-6aα, H-6′aα, H-3b, H-6b, H-6′b, H-3c, H-6c, H-6′c, H-3d, H-6d, H-6′d, H-3e, H-6e, H-6′e, H-5f, H-6f, H-6′f), 3.73–3.56 (m, 8H, H-5b, H-5c, H-5d, H-5e); ^{13}C NMR ($CDCl_3$): significant data for the α-anomer: δ 89.7 (C-1a), 76.3 (C-3a), 72.7 (C-2a), 70.2 (C-5a), 68.8 (C-4a); selected data connected with the β-anomer: δ 92.0 (C-1a), 78.3 (C-3a), 72.9 (C-5a), 72.5 (C-2a), 68.8 (C-4a), 62.7 (C6a); further chemical shifts: δ 166.0, 165.9, 165.8, 165.7, 165.4, 164.9, 164.7, 164.6, 164.4, 164.3, 164.1, 163.8, 163.6, 163.5 (OCOPh), 133.5, 133.2, 133.1, 133.0, 132.8, 132.6, 130.0, 129.9, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9 (C arom), 101.4, 101.0, 100.9, 100.8, 100.7 (C-1b, C-1c, C-1d, C-1e, C-1f), 78.3, 78.2, 78.1, 77.9 (C-3b, C-3c, C-3d, C-3e), 73.3, 73.2, 73.1, 72.9 (C-2), 72.5 (C-3f), 72.1 (C-5f), 71.7, 71.6 (C-5b, C-5c, C-5d, C-5e), 71.3 (C-2f), 70.2 (C-4), 70.0 (C-4), 69.8 (C-4f), 63.7, 63.4, 63.1 (C-6aα, C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 3093.8418 $[M+Na]^+$, calcd for $C_{176}H_{142}NaO_{51}$ 3093.8416.

4.3. Bromination of perbenzoylated oligo-β-(1,3)-glucans

To a solution of perbenzoylated glucan in CH_2Cl_2 cooled at 0 °C was added a 33 wt % solution of hydrobromic acid in acetic acid. After stirring for 3.5 h at room temperature, the media was diluted in CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$. The resulting organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure. The crude oil was subsequently used without purification.

4.3.1. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl bromide (4a)

This compound was synthesized starting from **3a** (444 mg, 0.170 mmol) and bromhydric acid in acetic acid (0.6 mL, 3.417 mmol), and isolated in a quantitative yield as a colorless oil (435 mg).

^{1}H NMR ($CDCl_3$): significant data: δ 6.55 (d, 1H, $J_{1a,2a}$ = 4.1 Hz, H-1a), 5.35 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 9.9 Hz, H-4a), 4.24 (dd, 1H, $J_{5a,6'a}$ = 4.6 Hz, $J_{6a,6'a}$ = 12.4 Hz, H-6′a); other signals: δ 8.92–7.61 (m, 22H, H arom), 7.48–6.93 (m, 58H, H arom), 5.27 (t, 1H, $J_{2e,3e} = J_{3e,4e}$ = 9.7 Hz, H-3e), 5.09 (dd, 1H, $J_{1e,2e}$ = 7.9 Hz, H-2e), 5.08 (t, 1H, $J_{4e,5e}$ = 9.7 Hz, H-4e), 4.93–4.86 (m, 3H, 2H-2, H-4), 4.80–4.70 (m, 4H, H-2a, H-1, 2H-4), 4.50–4.33 (m, 6H, H-3a, H-5a, H-6a, H-1e, 2H-1), 4.08–3.94 (m, 3H, H-5e, H-3, H-6), 3.87–3.70 (m, 9H, 2H-3, 7H-6), 3.65–3.53 (m, 3H, H-5b, H-5c, H-5d); ^{13}C NMR ($CDCl_3$): selected data: δ 87.7 (C-1a), 76.3 (C-3a), 73.4 (C-2a), 72.6 (C-5a), 67.6 (C-4a), 62.0 (C-6a); other signals: δ 165.9, 165.8, 165.7, 165.4, 164.9, 164.8, 164.7, 164.6, 164.5, 164.3, 163.8, 163.5 (OCOPh), 133.8, 133.4, 133.2, 133.1, 133.0, 132.9, 132.8, 132.6, 129.9, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9 (C arom), 101.3, 101.0, 100.8, 100.7 (C-1b, C-1c, C-1d, C-1e), 78.4, 78.1, 77.4 (C-3b, C-3c, C-3d), 73.3, 73.2, 72.7 (C-2b, C-2c, C-2d), 72.5 (C-3e), 71.8, 71.7, 71.6, 71.5 (C-5b, C-5c, C-5d, C-5e), 71.3 (C-2e), 70.2 (C-4b, C-4c, C-4d), 69.8 (C-4e), 63.4, 63.1 (C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2577.6011 $[M+Na]^+$, calcd for $C_{142}H_{115}BrNaO_{41}$ 2577.5995.

4.3.2. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl bromide (4b)

The title compound was prepared from **3b** (643 mg, 0.209 mmol) and HBr in AcOH (1.1 mL, 6.364 mmol) and obtained as a colorless oil in a quantitative yield (633 mg).

^{1}H NMR ($CDCl_3$): significant data: δ 6.68 (d, 1H, $J_{1a,2a}$ = 3.8 Hz, H-1a), 5.48 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 9.7 Hz, H-4a), 4.82 (dd, 1H, $J_{2a,3a}$ = 9.7 Hz, H-2a), 4.61 (t, 1H, H-3a), 4.60 (dd, 1H, $J_{5a,6a}$ = 3.8 Hz, $J_{6a,6'a}$ = 12.2 Hz, H-6a), 4.55–4.51 (m, 1H, H-5a), 4.37 (dd, 1H, $J_{5a,6'a}$ = 4.2 Hz, H6′a); other signals: δ 8.00–7.71 (m, 26H, H arom), 7.61–7.03 (m, 69H, H arom), 5.39 (t, 1H, $J_{2f,3f} = J_{3f,4f}$ = 9.7 Hz, H-3f), 5.22 (dd, 1H, $J_{1f,2f}$ = 7.8 Hz, H-2f), 5.21 (t, 1H, $J_{4f,5f}$ = 9.7 Hz, H-4f), 5.05–4.97 (m, 4H, 3H-2, H-4), 4.94 (t, 1H, J_{4-5} = 9.3 Hz, H-4),

4.88 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.87–4.77 (m, 3H, H-2, 2H-4), 4.55 (d, 1H, H-1f), 4.49 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.42 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.40 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.19–4.12 (m, 2H, H-3, H-6), 4.08–3.81 (m, 12H, 3H-3, 9H-6), 3.77–3.71 (m, 1H, H-5f), 3.71–3.60 (m, 4H, H-5b, H-5c, H-5d, H-5e); ^{13}C NMR ($CDCl_3$): selected data: δ 87.7 (C-1a), 76.3 (C-3a), 73.4 (C-2a), 72.7 (C-5a), 67.6 (C-4a), 62.0 (C-6a); other signals: δ 165.9, 165.8, 165.7, 165.4, 164.9, 164.8, 164.7, 164.6, 164.5, 164.3, 163.8, 163.5 (OCOPh), 133.8, 133.4, 133.2, 133.1, 133.0, 132.8, 132.6, 129.9, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9 (C arom), 101.3, 101.0, 100.8, 100.7 (C-1b, C-1c, C-1d, C-1e, C-1f), 78.4, 78.1, 77.8, 77.4 (C-3b, C-3c, C-3d, C-3e), 73.3, 73.2, 72.9 (C-2b, C-2c, C-2d, C-2e), 72.5 (C-3f), 71.8, 71.6 (C-5b, C-5c, C-5d, C-5e, C-5f), 71.3 (C-2f), 70.2 (C-4b, C-4c, C-4d, C-4e), 69.8 (C-4f), 63.5, 63.4, 63.1 (C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 3051.7334 $[M+Na]^+$, calcd for $C_{169}H_{137}BrNaO_{49}$ 3051.7310.

4.4. Synthesis of hexenopyranoses

To a solution of the crude oil previously obtained in CH_2Cl_2 was added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). It was further stirred for 5.5 h, diluted with CH_2Cl_2, and successively washed with a 10% aqueous solution of HCl, a saturated solution of $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure before chromatographic purification.

4.4.1. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-2-D-glu-hex-1-enopyranose (5a)

The pentasaccharidic derivative **5a** was obtained according to the general procedure starting from **4a** (435 mg, 0.170 mmol) in CH_2Cl_2 (10 mL) and using DBU (40 μL, 0.270 mmol). Chromatographic purification (3:2 petroleum ether/EtOAc) gave **5a** (343 mg, 81%) as a white solid.

R_f = 0.4 (3:2 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data: δ 6.63 (s, 1H, H-1a), 5.77 (d, 1H, $J_{3a,4a}$ = 3.3 Hz, H-3a), 4.73 (dd, 1H, $J_{5a,6a}$ = 9.0 Hz, $J_{6a,6'a}$ = 12.6 Hz, H-6a), 4.40 (dd, 1H, $J_{5a,6'a}$ = 3.1 Hz, H-6′a); other signals: δ 8.14–7.70 (m, 26H, H arom), 7.63–7.03 (m, 54H, H arom), 5.38 (t, 1H, $J_{2e,3e}$ = $J_{3e,4e}$ = 9.5 Hz, H-3e), 5.21 (dd, 1H, $J_{1e,2e}$ = 8.0 Hz, H-2e), 5.20 (t, 1H, H-4e, $J_{4e,5e}$ = 9.5 Hz), 5.17 (t, 1H, $J_{1b,2b}$ = $J_{2b,3b}$ = 8.8 Hz, H-2b), 5.12 (t, 1H, $J_{3b,4b}$ = $J_{4b,5b}$ = 10.5 Hz, H-4b), 5.05–4.97 (m, 2H, H-4c, H-2d), 4.94 (d, 1H, H-1b), 4.94–4.88 (m, 2H, H-2c, H-4d), 4.71 (d, 1H, $J_{1c,2c}$ = 6.7 Hz, H-1c), 4.59 (d, 1H, $J_{1e,2e}$ = 8.0 Hz, H-1e), 4.58–4.53 (m, 3H, H-4a, H-5a, H-1d), 4.46 (dd, 1H, $J_{5b,6b}$ = 3.1 Hz, $J_{6b,6'b}$ = 12.2 Hz, H-6b), 4.31 (dd, 1H, H-3b), 4.25 (dd, 1H, $J_{5b,6'b}$ = 6.6 Hz, H-6′b), 4.15–3.87 (m, 9H, H-5b, H-3c, H-6c, H-6′c, H-3d, H-6d, H-6′d, H-6e, H-6′e), 3.80 (dt, 1H, $J_{5d,6d}$ = 4.9 Hz, $J_{5d,6'd}$ = 9.3 Hz, H-5d), 3.76–3.68 (m, 2H, H-5c, H-5e); ^{13}C NMR ($CDCl_3$): selected data: δ 138.2 (C-1a), 74.1 (C-5a), 72.3 (C-4a), 68.7 (C-3a), 61.6 (C-6a); other signals: δ 165.9, 165.8, 165.7, 165.4, 165.3, 165.1, 165.0, 164.8, 164.7, 164.6, 163.9, 163.7 (OCOPh), 133.3, 133.2, 133.0, 132.9, 132.7, 130.1, 130.0, 129.9, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8 (C-2a, C arom), 101.2 (C-1b), 100.9 (C-1c), 100.8 (C-1e), 100.7 (C-1d), 78.2, 78.0, 77.7 (C-3b, C-3c, C-3d), 73.5 (C-2c), 73.2 (C-2b), 72.8 (C-2d), 72.7 (C-5b), 72.6 (C-3e), 72.1 (C-5d), 71.6 (C-5c, C-5e), 71.3 (C-2e), 70.3 (C-4c), 70.0 (C-4d), 69.9, 69.8 (C-4b, C-4e), 63.5, 63.4, 63.2 (C-6c, C-6d, C-6e), 63.1 (C-6b); HRMS found *m/z* 2497.6712 $[M+Na]^+$, calcd for $C_{142}H_{114}NaO_{49}$ 2497.6733.

4.4.2. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyran osyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-2-D-glu-hex-1-enopyranose (5b)

The hexasaccharidic derivative **5b** was prepared from **4b** (550 mg, 0.181 mmol) and DBU (35 mL, 0.235 mmol) in CH_2Cl_2 (10 mL). After flash chromatography eluting with 3:2 petroleum ether/EtOAc, a white solid was isolated (458 mg, 85%).

R_f = 0.5 (1:1 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data: δ 6.54 (s, 1H, H-1a), 5.69 (d, 1H, $J_{3a,4a}$ = 1.6 Hz, H-3a), 4.64 (dd, 1H, $J_{H5a-H6a}$ = 8.8 Hz, $J_{6a,6'a}$ = 12.4 Hz, H-6a), 4.32 (dd, 1H, $J_{5a,6'a}$ = 2.4 Hz, H-6′a); other signals: δ 7.95–7.62 (m, 25H, H arom), 7.51–6.93 (m, 70H, H arom), 5.29 (t, 1H, $J_{2f,3f}$ = $J_{3f,4f}$ = 9.7 Hz, H-3f), 5.12 (dd, 1H, $J_{1f,2f}$ = 8.0 Hz, H-2f), 5.11 (t, 1H, $J_{4f,5f}$ = 9.7 Hz, H-4f), 5.10–5.12 (m, 1H, H-2b, H-4b), 4.96–4.80 (m, 4H, H-1, 2H-2, H-4), 4.78–4.71 (m, 3H, H-2, 2H-4), 4.60 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.52–4.44 (m, 3H, H-4a, H-5a, H-1), 4.42–4.35 (m, 3H, 2H-1, H-6), 4.07–4.01 (m, 1H, H-5b), 3.98 (dd, 1H, $J_{5,6}$ = 3.5 Hz, $J_{6,6'}$ = 11.9 H, H-6z), 3.93–3.75 (m, 1H, 3H-3c, 8H-6), 3.74–3.55 (m, 4H, H-5c, H-5d, H-5e, H-5f); ^{13}C NMR ($CDCl_3$): selected data: δ 138.2 (C-1a), 74.1 (C-5a), 72.3 (C-4a), 68.6 (C-3a), 61.6 (C-6a); other signals: δ 165.8, 165.7, 165.4, 165.2, 165.1, 164.9, 164.7, 164.6, 163.8, 163.7, 163.6 (19C, OCOPh), 133.3, 133.2, 133.1, 133.0, 132.9, 132.8, 132.7, 132.6, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7 (C-2a, C arom), 101.2 (C-1b), 100.9, 100.8, 100.7 (C-1c, C-1d, C-1e, C-1f), 78.2, 78.0 (C-3b, C-3c, C-3d, C-3e), 73.3 (C-2), 73.1 (C-2b), 73.0 (C-2), 72.7 (C-5b), 72.6 (C-3f, C-2), 72.0, 71.8, 71.6 (C-5c, C-5d, C-5e, C-5f), 71.3 (C-2f), 70.2, 70.1, 69.8, 69.7 (C-4b, C-4c, C-4d, C-4e, C-4f), 63.5, 63.3, 63.1 (C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 2971.8034 $[M+Na]^+$, calcd for $C_{169}H_{136}NaO_{49}$ 2971.8048; HRMS found *m/z* 2987.7811 $[M+K]^+$, calcd for $C_{169}H_{136}KO_{49}$ 2987.7787.

4.5. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyran osyl-(1→3)-2,4,6-tri-*O*-benzoyl-2-*C*-bromo-α-D-mannopyran osyl bromide (6)

To a solution of glucal **5a** (90 mg, 0.036 mmol) in CH_2Cl_2 (0.5 mL) cooled at 0 °C were successively added molecular sieves (100 mg), ethanethiol (10 μL, 0.171 mmol), and *N*-bromosuccinimide (NBS, 8.5 mg, 0.048 mmol). After stirring for 30 min at 0 °C and 30 min at room temperature, the media was diluted (CH_2Cl_2, 20 mL), washed with a 10% aqueous solution of $Na_2S_2O_4$, and finally with cooled brine. The solvent was then dried ($MgSO_4$), removed under reduced pressure to afford the target dibromide derivative **6** (96 mg, 100%).

^{1}H NMR ($CDCl_3$): significant data: δ 5.81 (dd, 1H, $J_{3a,4a}$ = 9.3 Hz, $J_{4a,5a}$ = 10.0 Hz,, H-4a), 4.62–4.54 (m, 1H, H-5a), 4.43 (dd, 1H, $J_{5a,6a}$ = 2.5 Hz, $J_{6a,6'a}$ = 12.8 Hz,, H-6a), 4.24 (dd, 1H, $J_{5a,6'a}$ = 3.3 Hz, H-6′a); other signals: δ 8.06–7.69 (m, 32H, H arom), 7.60–7.10 (m, 49H, H-1a, H arom), 5.40 (t, 1H, $J_{2e,3e}$ = $J_{3e,4e}$ = 9.7 Hz, H-3e), 5.24–5.16 (m, 5H, H-1b, H-2b, H-4b, H-2e, H-4e), 5.06–4.98 (m, 2H, H-4c, H-2d), 4.93–4.84 (m, 3H, H-3a, H-2c, H-4d), 4.66 (d, 1H, $J_{H1c-H2c}$ = 8.0 Hz, H-1c), 4.59 (d, 1H, $J_{1e,2e}$ = 8.0 Hz, H-1e), 4.51 (d, 1H, $J_{1d,2d}$ = 8.0 Hz, H-1d), 4.35–4.26 (m, 1H, H-3b), 4.07–3.64 (m, 14H, H-5b, H-6b, H-6′b, H-3c, H-5c, H-6c, H-6′c, H-3d, H-5d, H-6d, H-6′d, H-5e, H-6e, H-6′e); ^{13}C NMR ($CDCl_3$): selected data: δ 89.9 (C-2a), 87.8 (C-1a), 79.5 (C-3a), 72.9 (C-5a), 67.3 (C-4a), 61.6 (C-6a); other signals: δ 166.6, 165.8, 165.7, 165.4, 164.9, 164.8, 164.7, 164.6, 164.5, 163.9, 163.6, 163.4, 162.5 (16C–, OCOPh), 133.3, 133.1, 133.0, 132.9, 132.8, 130.4, 130.3, 130.0,

129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.8, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6 (C arom), 101.6 (C-1b), 101.0 (C-1c), 100.9 (C-1e), 100.7 (C-1d), 78.1 (C-3b, C-3c, C-3d), 73.5 (C-2c), 73.1 (C-2b), 72.9 (C-2d), 72.6 (C-3e), 71.6, 71.3 (C-5b, C-5c, C-5d, C-2e, C-5e), 70.1 (C-4c, C-4d), 69.8 (C-4b, C-4e), 63.6, 63.4, 63.3, 63.1 (4C, C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2655.5017 $[M+Na]^+$, calcd for $C_{142}H_{114}Br_2NaO_{41}$ 2655.5101; HRMS found *m/z* 2671.4854 $[M+K]^+$, calcd for $C_{169}H_{136}$ Br_2KO_{49} 2671.4839.

4.6. Synthesis of ulosyl bromides

A solution of hexenopyranose in MeOH was stirred at room temperature for 10 min and cooled at 0 °C. *N*-Bromosuccinimide (NBS) was then added and the reaction monitored by TLC. After completion of the reaction (2 h), the media was diluted with CH_2Cl_2, successively washed with an aqueous saturated solution of $Na_2S_2O_3$ and water, dried ($MgSO_4$), and concentrated. The resulting crude oil was used without further purification for the following step.

4.6.1. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranos-2-ulosyl bromide (7a)

This compound was obtained starting from **5a** (48 mg, 0.019 mmol) diluted in CH_2Cl_2 (5 mL) and anhydrous MeOH (1.6 μL, 0.040 mmol), and NBS (10.8 mg, 0.060 mmol). Removal of the solvent afforded **7a** (47 mg) in a quantitative yield as a colorless oil.

^{1}H NMR ($CDCl_3$): significant data: δ 6.25 (s, 1H, H-1a), 5.44 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 10.1 Hz, H-4a), 5.17 (d, 1H, H-3a); other signals: δ 8.06–7.71 (m, 20H, H arom), 7.61–7.12 (m, 55H, H arom), 5.39 (t, 1H, $J_{2e,3e} = J_{3e,4e}$ = 9.5 Hz, H-3e), 5.22 (dd, 1H, $J_{1e,2e}$ = 8.0 Hz, H-2e), 5.21 (t, 1H, $J_{4e,5e}$ = 9.5 Hz, H-4e), 5.10 (dd, 1H, $J_{1b,2b}$ = 8.2 Hz, $J_{2b,3b}$ = 9.3 Hz, H-2b), 5.06–4.98 (m, 4H, H-1b, H-4b, H-4c, H-2d), 4.89 (t, 1H, $J_{3d,4d} = J_{4d,5d}$ = 9.5 Hz, H-4d), 4.88 (dd, 1H, $J_{1c,2c}$ = 7.7 Hz, $J_{2c,3c}$ = 8.6 Hz, H-2c), 4.74 (d, 1H, $J_{1c,2c}$ = 8.2 Hz, H-1c), 4.69 (d, 1H, H-1e), 4.66–4.55 (m, 3H, H-5a, H-6a, H-1d), 4.39–4.26 (m, 2H, H-3b, H-6), 4.24–4.10 (m, 3H, 3H-6), 4.05–3.86 (m, 8H, H-5b, H-3c, H-3d, 5H-6), 3.82–3.70 (m, 3H, H-5c, H-5d, H-5e); ^{13}C NMR ($CDCl_3$): significant data: δ 191.5 (C-2a), 83.8 (C-1a), 76.3 (C-3a), 72.6 (C-5a), 69.0 (C-4a), 61.7 (C-6a); other signals: δ 167.1, 165.9, 165.8, 165.7, 165.4, 164.9, 164.7, 164.6, 164.3, 164.1, 163.9, 163.6 (OCOPh), 133.6, 133.3, 133.2, 133.1, 133.0, 132.9, 132.8, 132.7, 130.3, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6 (C arom), 101.0, 100.9, 100.7, 100.6 (C-1b, C-1c, C-1d, C-1e), 78.2, 78.0, 77.7 (C-3b, C-3c, C-3d), 73.6, 73.1, 72.8 (C-2b, C-2c, C-2d), 72.6 (C-3e), 71.9, 71.6 (C-5b, C-5c, C-5d, C-5e), 71.3 (C-2e), 70.1, 69.9, 69.8 (C-4b, C-4c, C-4d, C-4e), 63.5, 63.4, 63.1 (C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2471.5595 $[M+Na]^+$, calcd for $C_{135}H_{109}BrNaO_{40}$ 2471.5576.

4.6.2. 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranos-2-ulosyl bromide (7b)

As described in the general procedure, compound **7b** was synthesized from **5b** (124 mg, 0.042 mmol), anhydrous MeOH (3.4 μL, 0.084 mmol), CH_2Cl_2 (1.5 mL), NBS (22.4 mg, 0.126 mmol), and obtained as a colorless oil in a quantitative yield (123 mg).

^{1}H NMR ($CDCl_3$): significant data: δ 6.25 (s, 1H, H-1a), 5.44 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 10.2 Hz, H-4a), 5.16 (d, 1H, H-3a); other signals: δ 8.09–7.70 (m, 25H, H arom), 7.60–6.84 (m, 65H, H arom), 5.37 (t, 1H, $J_{2f,3f} = J_{3f,4f}$ = 9.7 Hz, H-3f), 5.20 (dd, 1H, $J_{1f,2f}$ = 8.0 Hz, H-2f), 5.19 (t, 1H, $J_{4f,5f}$ = 9.7 Hz, H-4f), 5.10 (dd, 1H, $J_{1b,2b}$ = 8.1 Hz, $J_{2b,3b}$ = 9.1 Hz, H-2b), 5.07–4.96 (m, 4H, H-1b, H-2, 2H-4), 4.92–4.78 (m, 4H, 2H-2, 2H-4), 4.73 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.66 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.65–4.57 (m, 2H, H-5a, H-6), 4.54 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.49 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.47–4.41 (m, 1H, H-6), 4.35 (dd, 1H, $J_{5,6}$ = 4.9 Hz, $J_{6,6'}$ = 12.4 Hz, H-6), 4.28 (t, 1H, $J_{3b,4b}$ = 9.1 Hz, H-3b), 4.19 (dd, 1H, $J_{5,6}$ = 3.5 Hz, $J_{6,6'}$ = 12.2 Hz, H-6), 4.12 (dd, 1H, $J_{5,6}$ = 6.2 Hz, $J_{66'}$ = 12.2 Hz, H-6), 4.05–3.81 (m, 11H, H5-b, H-3c, H-3d, H-3e, 7H-6), 3.80–3.62 (m, 4H, H-5c, H-5d, H-5e, H-5f); ^{13}C NMR ($CDCl_3$): significant data: δ 191.5 (C-2a), 83.9 (C-1a), 76.3 (C-3a), 72.6 (C-5a), 69.0 (C-4a), 61.7 (C-6a); other signals: δ 165.9, 165.8, 165.7, 165.4, 164.9, 164.7, 164.6, 164.3, 163.8, 163.6 (OCOPh), 133.6, 133.3, 133.2, 133.1, 133.0, 132.9, 132.6, 130.0, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 127.9 (C arom), 101.0, 100.9, 100.7, 100.6 (C-1b, C-1c, C-1d, C-1e, C-1f), 78.3, 78.2, 78.1, 78.0 (C-3b, C-3c, C-3d, C-3e), 73.5, 73.1, 73.0, 72.8 (C-2b, C-2c, C-2d, C-2e), 72.6 (C-3f), 71.9, 71.8, 71.6 (C-5b, C-5c, C-5d, C-5e, C-5f), 71.3 (C-2f), 70.2, 70.1, 70.0, 69.8 (C-4b, C-4c, C-4d, C-4e, C-4f), 63.6, 63.5, 63.4, 63.3, 63.2 (C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 2945.6917 $[M+Na]^+$, calcd for $C_{162}H_{131}BrNaO_{48}$ 2945.6891.

4.7. Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-6-*O*-benzoyl-4-deoxy-β-D-gluco-hex-3-enopyranos-2-uloside (8)

To a solution of ulosyl bromide **7a** (50 mg, 0.020 mmol) in CH_2Cl_2 (0.75 mL) were successively added at 0 °C benzyl alcohol (4.4 μL, 0.040 mmol) and silver triflate (5.2 mg, 0.020 mmol). After stirring for 3 h at room temperature, the reaction media was neutralized by adding few drops of triethylamine, and then filtered over a bed of Celite. The concentrated substrate was finally purified by flash chromatography eluting with 3:2 petroleum ether/EtOAc to give the enone **8** (38 mg, 75%).

An alternative procedure consisted in substituting silver triflate by silver carbonate. To a suspension of benzyl alcohol (4.4 μL, 0.040 mmol), silver carbonate (11.6 mg, 0.042 mmol) and molecular sieves (50 mg) in dichloromethane (1 mL) stirred for 15 min was further added ulosyl bromide **7a** (50 mg, 0.020 mmol). The glycosylation was performed for 2 h at room temperature and filtered over a bed of Celite. Subsequent purification as indicated previously afforded the enone **8** in a similar yield (38 mg, 75%).

R_f = 0.5 (1:1 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data: δ 6.26 (d, 1H, H-4a, $J_{4a,5a}$ = 3.6 Hz, H-4a), 4.87 (s, 1H, H-1a, 4.83 (d, 1H, $J_{7a,7'a}$ = 11.7 Hz, H-7a), 4.68–4.61 (m, 1H, H-5a), 4.60 (d, 1H, H-7′a); Complementary signals: δ 8.10–7.70 (m, 20H, H arom), 7.60–7.12 (m, 55H, H arom), 5.39 (t, 1H, $J_{2e,3e} = J_{3e,4e}$ = 9.7 Hz, H-3e), 5.28–5.17 (m, 5H, H-1b, H-2b, H-4b, H-2e, H-4e), 5.09–5.02 (m, 4H, H-2c, H-4c, H-2d, H-4d), 4.95 (d, 1H, $J_{1c,2c}$ = 8.0 Hz, H-1c), 4.63 (d, 1H, $J_{1d,2d}$ = 7.9 Hz, H-1d), 4.61 (d, 1H, $J_{1e,2e}$ = 8.0 Hz, H-1e), 4.53–4.40 (m, 3H, H-6a, 2H-6), 4.38–4.32 (m, 2H, H-3b, H-6), 4.25–4.18 (m, 2H, H-6′a, H-3), 4.15–3.90 (m, 8H, H-5b, H-3, H-5, 5H-6), 3.80–3.70 (m, 2H, H-5e, H-5); ^{13}C NMR ($CDCl_3$): significant data: δ 183.5 (C-2a), 144.9 (C-3a), 126.2 (C-4a), 97.7 (C-1a), 71.0 (C-5a), 70.6 (C-7a), 66.1 (C-6a); other signals: δ 165.8, 165.7, 165.4, 164.9, 164.6, 164.5, 163.9, 163.8 (OCOPh), 136.1 (C quat. arom OCH_2*Ph*), 133.3, 133.2, 133.1, 133.0, 132.9, 132.7, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8 (C arom), 100.9 (C-1d, C-1e), 100.1 (C-1c), 98.3 (C-1b), 78.3, 78.0 (C-3c, C-3d), 76.8 (C-3b), 73.7 (C-2c, C-2d),

73.0 (C-2b), 72.8 (C-5b), 72.6 (C-3e), 72.1 (C-5), 71.6 (C-2e), 71.3 (C-5e, C-5), 69.9, 69.8 (C-4c, C-4d, C-4e), 69.3 (C-4b), 63.4 (C-6e, C-6), 63.1 (C-6b, C-6); HRMS found *m/z* 2377.6516 $[M+Na]^+$, calcd for $C_{1359}H_{110}NaO_{39}$ 2377.6522.

4.8. Glycosylation of benzyl alcohol with ulosyl bromide as donor

A suspension of ulosyl bromide, benzyl alcohol and molecular sieves in CH_2Cl_2 was vigorously stirred for 5 min at room temperature. Triphenylphosphine oxide was then added. After 4 days, the molecular sieves were removed by filtration over Celite, and the organic layer concentrated under reduced pressure. A flash chromatography afforded the desired glycoside in its hydrated form.

4.8.1. Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzoyl-2-C-hydroxyl-β-D-glucopyranoside (9a)

Compound **9a** was prepared from **7a** (94 mg, 0.038 mmol), benzyl alcohol (8 μL, 0.077 mmol), triphenylphosphine oxide (21 mg, 0.077 mmol) in CH_2Cl_2 (1.5 mL). A chromatographic purification (3:2 petroleum ether/EtOAc) gave the target pentasaccharide **9a** (83 mg, 87%) as a colorless oil.

R_f = 0.5 (1:1 petroleum ether/EtOAc); ^1H NMR ($CDCl_3$): significant data: δ 4.45–4.34 (m, 3H, H-5a, H-6a, H-6′a), 4.25 (s, 1H, H-1a); other signals: δ 8.00–7.10 (m, 80H, H arom), 5.42–5.16 (m, 5H, H-4a, H-2e, H-3e, H-4e, H-2), 5.12–4.79 (m, 6H, H-7a, H-4b, H-4c, H-4d, 2H-2), 4.78–4.68 (m, 3H, H-7′a, 2H-1), 4.64–4.46 (m, 2H, 2H-1), 4.26 (t, 1H, $J_{2b,3b} = J_{3b,4b}$ = 9.0 Hz, H-3b), 4.15–3.69 (m, 15H, H-3a, H-5b, H-6b, H-6′b, H-3c, H-5c, H-6c, H-6′c, H-3d, H-5d, H-6d, H-6′d, H-5e, H-6e, H-6′e); ^{13}C NMR ($CDCl_3$): significant data: δ 98.7 (C-1a, $^1J_{H1a,C1a}$ = 168 Hz), 93.0 (C-2a), 81.7 (C-3a), 70.7 (C-7a), 69.3 (C-4a), 63.1 (C-6a); other signals: δ 166.1, 165.8, 165.7, 165.4, 165.0, 164.9, 164.7, 164.2, 163.8, 163.7, 163.6 (OCOPh), 133.4, 133.2, 133.1, 133.0, 132.7, 130.0, 129.9, 129.7, 129.6, 129.5, 129.3, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0 (C arom), 102.4 (C-1b), 101.0, 100.9 (C-1c, C-1d, C-1e), 78.2, 78.0, 77.7 (C-3b, C-3c, C-3d), 73.4, 72.8, 72.6 (C-5a, C-2b, C-2c, C-2d, C-3e), 71.8, 71.6 (C-5b, C-5c, C-5d, C-5e), 71.3 (C-2e), 70.2, 70.0, 69.9, 69.7 (C-4b, C-4c, C-4d, C-4e), 63.6, 63.5, 63.4 (C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2499.6898 $[M+Na]^+$, calcd for $C_{142}H_{116}NaO_{41}$ 2499.6890; HRMS found *m/z* 2517.6991 $[M+K]^+$, calcd for $C_{142}H_{118}KO_{42}$ 2517.6991.

4.8.2. Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzoyl-2-C-hydroxyl-β-D-glucopyranoside (9b)

Synthesis of compound **9b** required **7b** (51 mg, 0.017 mmol), CH_2Cl_2 (0.7 mL), benzyl alcohol (3.6 μL, 0.035 mmol), triphenylphosphine oxide (9.7 mg, 0.035 mmol). A flash chromatography eluting with 3:2 petroleum ether/EtOAc gave **9b** as a colorless oil (43.8 mg, 85%).

R_f = 0.3 (3:2 petroleum ether/EtOAc); ^1H NMR ($CDCl_3$): significant data: δ 4.61 (d, 1H, $J_{7a,7'a}$ = 12.2 Hz, H-7′a), 4.57–4.47 (m, 1H, H-6a), 4.25 (s, 1H, H-1a); complementary data: δ 8.03–7.69 (m, 25H, H arom), 7.58–7.08 (m, 70H, H arom), 5.36 (t, 1H, $J_{2f,3f}$ = 9.7 Hz, H-3f), 5.30–5.10 (m, 3H, H-2f, H-4a, H-4f), 5.08–4.94 (m, 4H, 2H-2, 2H-4), 4.92–4.73 (m, 5H, H-7a, 2H-2, 2H-4), 4.45–4.32 (m, 5H, H-5a, H-6′a, 2H-1), 4.24–4.19 (m, 1H, H-3b), 4.15–3.77 (m, 14H, H-3a, H-3c, H-3d, H-3e, H-6b, H-6′b, H-6c, H-6′c, H-6d, H-6′d, H-6e, H-6′e, H-6f, H-6′f), 3.74–3.65 (m, 5H, H-5b, H-5c, H-5d, H-5e, H-5f); ^{13}C NMR ($CDCl_3$): significant data: δ 98.7 (C-1a, $^1J_{H1a,C1a}$ = 171 Hz), 93.0 (C-2a), 81.7 (C-3a), 70.7 (C-7a), 69.8 (C-4a); complementary data: δ 166.1, 165.8, 165.7, 165.4, 164.9, 164.8, 164.7, 164.6, 163.8, 163.6 (OCOPh), 133.4, 133.3, 133.2, 133.1, 133.0, 132.6, 132.1, 132.0, 129.9, 129.8, 129.6, 129.4, 129.3, 129.2, 129.1, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6, 126.9, 126.5 (C arom), 102.4 (C-1b), 100.9, 100.8 (C-1c, C-1d, C-1e, C-1f), 78.3, 78.2, 78.1 (C-3b, C-3c, C-3d, C-3e), 73.1, 73.0 (C-2), 72.6 (C-3f, C-5a, 2 C-2), 71.8, 71.6 (C-5b, C-5c, C-5d, C-5e, C-5f), 71.3 (C-2f), 70.3, 70.2, 70.1, 69.9 (C-4b, C-4c, C-4d, C-4e, C-4f), 63.6, 63.5, 63.4, 63.2, 63.1 (C-6a, C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 2973.8163 $[M+Na]^+$, calcd for $C_{169}H_{138}NaO_{49}$ 2973.8204; HRMS found *m/z* 2991.8106 $[M+Na]^+$, calcd for $C_{169}H_{140}KO_{50}$ 2991.8310.

4.9. Reduction with L-Selectride

To a solution of benzylic glycoside in THF cooled at −78 °C was added L-Selectride. After stirring for 1.5 h at the same temperature, the reaction was quenched by adding few drops of HOAc. The media was further diluted with CH_2Cl_2, successively washed with a 10% aqueous solution of HCl, a saturated aqueous solution of $NaHCO_3$, and finally with brine. The desired glucanyl-mannose derivative was then obtained by drying the organic layer ($MgSO_4$) and removal of the solvent.

4.9.1. Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzoyl-β-D-mannopyranoside (10a)

Reduction of **9a** (83 mg, 0.034 mmol) required L-Selectride (1 M in THF, 34 μL, 0.034 mmol) in THF (0.7 mL). Pentasaccharide **10a** was isolated in a quantitative yield (83 mg) as a colorless oil.

R_f = 0.5 (1:1 petroleum ether/EtOAc); ^1H NMR ($CDCl_3$): significant data: δ 5.53 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 7.3 Hz, H-4a), 4.82 (d, 1H, $J_{7a,7'a}$ = 12.4 Hz, H-7a), 4.49 (d, 1H, $J_{1a,2a}$ = 2.0 Hz, H-1a); Complementary signals: δ 8.04–7.10 (m, 80H, H arom), 5.41 (t, 1H, $J_{2e,3e} = J_{3e,4e}$ = 9.5 Hz, H-3e), 5.24 (dd, 1H, $J_{1e,2e}$ = 8.0 Hz, H-2e), 5.22 (t, 1H, $J_{4e,5e}$ = 9.5 Hz, H-4e), 5.15–4.85 (m, 6H, H-2b, H-4b, H-2c, H-4c, H-2d, H-4d), 4.76 (d, 1H, $J_{1b,2b}$ = 7.7 Hz, H-1b), 4.65–4.50 (m, 6H, H-7′a, H-6a, H-6′a, H-1c, H-1d, H-1e), 4.34–3.83 (m, 14H, H-2a, H-3a, H-3b, H-6b, H-6′b, H-3c, H-6c, H-6′c, H-3d, H-6d, H-6′d, H-6e, H-6′e, 2H-5), 3.80–3.71 (m, 3H, 3H-5); ^{13}C NMR ($CDCl_3$): significant data: δ 96.5 (C-1a), 70.1 (C-7a), 68.4 (C-4a), 67.1 (C-2a), 64.1 (C-6a); complementary data: δ 166.0, 165.8, 165.7, 165.4, 165.0, 164.9, 164.7, 164.6, 164.1, 163.8, 163.6 (OCOPh), 136.3 (C quat arom OCH_2*Ph*), 133.2, 133.1, 133.0, 132.9, 132.6, 129.9, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7 (C arom), 100.8, 100.7, 100.6 (C-1b, C-1c, C-1d, C-1e), 78.1, 78.0, 77.7 (C-3a, C-3b, C-3c, C-3d), 73.6, 73.5, 72.8 (C-2b, C-2c, C-2d) 72.5 (C-3e), 72.2, 72.0, 71.9, 71.6 (C-5a, C-5b, C-5c, C-5d, C-5e), 71.3 (C-2e), 70.0, 69.8, 69.7 (C-4b, C-4c, C-4d, C-4e), 63.4, 63.2, 63.1, 62.7 (C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 2501.7064 $[M+Na]^+$, calcd for $C_{142}H_{118}NaO_{49}$ 2501.7046; HRMS found *m/z* 2517.6991 $[M+K]^+$, calcd for $C_{142}H_{118}KO_{41}$ 2517.6786.

4.9.2. Benzyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzoyl-β-D-mannopyranoside (10b)

Reduction of **9b** (83 mg, 0.034 mmol) required L-Selectride (1 M in THF, 18 μL, 0.018 mmol) in THF (0.5 mL). Hexasaccharide **10b** was isolated in 98% yield (50 mg) as a colorless oil.

R_f = 0.5 (1:1 petroleum ether/EtOAc); ^{1}H NMR ($CDCl_3$): significant data: δ 5.48 (t, 1H, $J_{3a,4a} = J_{4a,5a}$ = 7.3 Hz, H-4a), 4.82 (d, 1H, $J_{7a,7'a}$ = 12.4 Hz, H-7a), 4.45 (d, 1H, $J_{1a,2a}$ = 2.2 Hz, H-1a); other signals: δ 8.00–7.70 (m, 25H, H arom), 7.55–7.10 (m, 90H, H arom), 5.35 (t, 1H, $J_{2f,3f} = J_{3f,4f}$ = 9.5 Hz, H-3f), 5.17 (dd, 1H, $J_{1f,2f}$ = 8.0 Hz, H-2f), 5.16 (t, 1H, $J_{4f,5f}$ = 9.5 Hz, H-4f), 5.15–5.10 (m, 1H, H-4), 5.08 (dd, 1H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 9.1 Hz, H-2), 5.02–4.90 (m, 3H, H-2, 2H-4d), 4.89–4.75 (m, 3H, 2H-2, H-4), 4.71 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.68 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.57–4.36 (m, 6H, H-7′a, 3H-1, 2H-6), 4.32–4.19 (m, 2H, H-3, H-6), 3.99–3.78 (m, 16H, H-2a, 4H-3, 2H-5, 9H-6), 3.76–3.63 (m, 4H, 4H-5); ^{13}C NMR ($CDCl_3$): significant data: δ 96.6 (C-1a), 69.8 (C-7a), 68.5 (C-4a), 67.1 (C-2a), 64.2 (C-6a); complementary data: δ 165.8, 165.7, 165.4, 165.1, 164.9, 164.7, 164.1, 163.8, 163.6 (OCOPh), 136.4 (C quat arom OCH_2*Ph*), 133.2, 133.1, 133.0, 132.6, 130.0, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 129.0, 128.8, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9 (C arom), 100.9, 100.8 (C-1b, C-1c, C-1d, C-1e, C-1f), 78.4, 78.3, 78.2, 78.0 (C-3a, C-3b, C-3c, C-3d, C-3e), 73.6, 73.5, 73.3 (C-2), 73.0 (C-3f, C-2), 72.2, 72.0, 71.7, 71.6 (C-5a, C-5b, C-5c, C-5d, C-5e, C-5f), 71.3 (C-2f), 70.2, 70.1 (C-4b, C-4c, C-4d, C-4e), 69.3 (C-4f), 63.6, 63.5, 63.3, 63.2 (C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 2975.8366 $[M+Na]^+$, calcd for $C_{169}H_{140}NaO_{49}$ 2975.8361.

4.10. Zemplén debenzoylation

The oil previously obtained was then diluted in a 2:1 mixture of HOMe/CH_2Cl_2 containing 2 equiv of NaOMe (0.1 M MeONa in HOMe). After stirring for 6 h at room temperature, neutralization was performed with the help of Amberlite IR 120 (H^+-form). The resin was further filtered off, and the solvent removed under reduced pressure. The oligosaccharide was finally purified by flash chromatography (99:1 CH_2Cl_2/HOMe).

4.10.1. Benzyl β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-mannopyranoside (11a)

Deacylation of **10a** (83 mg, 0.034 mmol) in NaOMe, HOMe (0.1 M solution, 0.7 mL, 0.070 mmol) afforded **11a** (28 mg) in 91% yield as a colorless oil.

^{1}H NMR ($CDCl_3$): significant data: δ 5.03 (d, 1H, $J_{1a,2a}$ = 3.6 Hz, H-1a), 4.81 (d, 1H, $J_{7a,7'a}$ = 12.2 Hz, H-7a), 4.58 (d, 1H, H-7′a); complementary signals: δ 7.88–7.83 (m, 2H, H arom), 7.33–7.13 (m, 3H, H arom), 4.56 (d, 2H, $J_{1,2}$ = 8.0 Hz, 2H-1), 4.46 (d, 2H, $J_{1,2}$ = 7.7 Hz, 2H-1), 3.3–3.4 (m, 7H, H-3a, H-4a, H-6a, H-6b, H-6c, H-6d, H-6e), 3.64–3.46 (m, 10H, H-2a, H-6′a, H-3b, H-6′b, H-3c, H-6′c, H-3d, H-6′d, H-5e, H-6′e), 3.44–3.34 (m, 3H, H-2b, H-2c, H-2d), 3.33–3.13 (m, 10H, H-5a, H-4b, H-5b, H-4c, H-5c, H-4d, H-5d, H-2e, H-3e, H-4e); ^{13}C NMR ($CDCl_3$): significant data: δ 99.9 (C-1a), 85.3 (C-3a), 73.1 (C-2a), 72.9 (C-4a), 71.5 (C-7a); complementary data: δ 131.0 (C quat arom), 129.4, 128.9 (C arom), 105.1, 104.7 (C-1b, C-1c, C-1d, C-1e), 87.4, 87.1 (C-3b, C-3c, C-3d), 82.1 (C-5e), 78.1, 77.7 (C-5a, C-5b, C-5c, C-5d, C-3e), 75.5 (C-2e), 75.0, 74.9 (C-2b, C-2c, C-2d), 71.6 (C-4e), 70.0, 69.9 (C-4b, C-4c, C-4d), 62.6, 62.5 (C-6a, C-6b, C-6c, C-6d, C-6e); HRMS found *m/z* 941.3119 $[M+Na]^+$, calcd for $C_{37}H_{58}NaO_{26}$ 941.3114; HRMS found *m/z* 957.2892 $[M+K]^+$, calcd for $C_{37}H_{58}KO_{26}$ 957.2853.

4.10.2. Benzyl β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-mannopyranoside (11b)

Deacylation was performed from **10b** (44 mg, 0.015 mmol) and NaOMe, HOMe (0.1 M, 0.3 mL, 0.030 mmol) and gave the hexasaccharide **11b** (15.7 mg, 96%) as a colorless oil.

^{1}H NMR ($CDCl_3$): significant data: δ 4.95 (d, 1H, $J_{1a,2a}$ = 3.8 Hz, H-1a), 4.75 (d, 1H, $J_{7a,7'a}$ = 12.2 Hz, H-7a), 4.50 (d, 1H, H-7′a); other signals: δ: 7.22–7.10 (m, 5H, H arom), 4.48 (d, 3H, $J_{1,2}$ = 8.0 Hz, 3H-1), 4.39 (d, 2H, $J_{1,2}$ = 7.5 Hz, 2H-1), 3.76–3.53 (m, 8H, H-3a, H-4a, H-6a, H-6b, H-6c, H-6d, H-6e, H-6f), 3.55–3.36 (m, 12H, H-2a, H-3b, H-3c, H-3d, H-3e, H-5f, H-6′a, H-6′b, H-6′c, H-6′d, H-6′e, H-6′f), 3.35–3.25 (m, 4H, H-2b, H-2c, H-2d, H-2e), 3.24–3.05 (m, 12H, H-2f, H-3f, H-4b, H-4c, H-4d, H-4e, H-4f, H-5a, H-5b, H-5c, H-5d, H-5e); ^{13}C NMR ($CDCl_3$): significant data: δ 99.9 (C-1a), 85.3 (C-3a), 73.1 (C-2a), 72.2 (C-4a), 71.7 (C-7a); complementary data: δ 131.2 (C quat arom), 129.4, 128.7 (C arom), 105.2, 104.7 (C-1b, C-1c, C-1d, C-1e, C-1f), 87.4, 87.1, 87.0 (C-3b, C-3c, C-3d, C-3e), 82.1 (C-5f), 78.2, 78.1, 77.8 (C-3f, C-5a, C-5b, C-5c, C-5d, C-5e), 75.5 (C-2f), 75.0, 74.9, 74.4 (C-2b, C-2c, C-2d, C-2e), 71.5 (C-4f), 70.0, 69.9 (C-4b, C-4c, C-4d, C-4e), 62.8, 62.6, 62.5 (C-6a, C-6b, C-6c, C-6d, C-6e, C-6f); HRMS found *m/z* 1103.3639 $[M+Na]^+$, calcd for $C_{43}H_{68}NaO_{31}$ 1119.3338; HRMS found *m/z* 957.2892 $[M+K]^+$, calcd for $C_{43}H_{68}KO_{31}$ 1119.3382.

4.11. Hydrolytic debenzylation

To a solution of benzyl oligoglucan in methanol was added $Pd(OAc)_2$. After stirring for 7 days at room temperature under hydrogen atmosphere, the catalyst was removed by filtration and the solvent under reduced pressure. Elution on a Sephadex G-10 column eluting with water, followed by freeze drying, yield the desired glucan-mannose conjugate.

4.11.1. β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α,β-D-mannopyranose (1a)

Debenzylation of **11a** (25.2 mg, 0.027 mmol) in the presence of $Pd(OAc)_2$ quantitatively gave **1a** (22.5 mg) as a white foam.

R_f = 0.2 (3:2:2 EtOAc/HO*i*Pr/H_2O); ^{1}H NMR ($CDCl_3$): significant data: δ 5.20 (d, 1H, $J_{1a,2a}$ = 1.8 Hz, H-1aα); complementary signals: δ 4.75 (d, 2H, $J_{1,2}$ = 8.2 Hz, 2H-1), 4.71 (d, 2H, $J_{1,2}$ = 8.0 Hz, 2H-1,; 3.91–3.80 (m, 7H, H-3a, H-4a, H-6a, H-6b, H-6c, H-6d, H-6e), 3.78–3.59 (m, 9H, H-2a, H-6′a, H-3b, H-6′b, H-3c, H-6′c, H-3d, H-6′d, H-6′e), 3.58–3.40 (m, 12H, H-5a, H-2b, H-4b, H-5b, H-2c, H-4c, H-5c, H-2d, H-4d, H-5d, H-3e, H-5e), 3.36 (t, 1H, $J_{3e,4e} = J_{4e,5e}$ = 9.7 Hz, H-4e), 3.31 (dd, 1H, $J_{1e,2e}$ = 8.0 Hz, $J_{2e,3e}$ = 9.3 Hz, H-2e; HRMS found *m/z* 851.2634 $[M+Na]^+$, calcd for $C_{303}H_{52}NaO_{26}$ 851.2644.

4.11.2. β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-α,β-D-mannopyranose (1b)

Debenzylation of **11b** (15.7 mg, 0.015 mmol) in the presence of $Pd(OAc)_2$ (16 mg, 0.071 mmol) afforded the desired hexasaccharide **1b** (14.3 mg, 100%) as a white foam.

R_f = 0.2 (3:2:2 EtOAc/HO*i*Pr/H_2O); ^{1}H NMR ($CDCl_3$): significant data: 5.19 (d, 1H, $J_{1a,2a}$ = 2.2 Hz, H-1aα); complementary signals: δ 4.75 (d, 3H, $J_{1,2}$ = 8.0 Hz, 3H-1), 4.71 (d, 2H, $J_{1,2}$ = 7.8 Hz, 2H-1), 3.93–3.81 (m, 8H, H-3a, H-4a, H-6a, H-6b, H-6c, H-6d, H-6e, H-6f), 3.80–3.63 (m, 11H, H-2a, H-3b, H-3c, H-3d, H-3e, H-6′a, H-6′b, H-6′c, H-6′d, H-6′e, H-6′f), 3.55–3.40 (m, 11H, H-2b, H-2c, H-2d, H-2e, H-3f, H-4b, H-4c, H-4d, H-4e, H-5a, H-5b, H-5c, H-5d, H-5e, H-5f), 3.36 (t, 1H, $J_{3f,4f} = J_{4f,5f}$ = 9.3 Hz, H-4f), 3.31 (dd, 1H, $J_{1f,2f}$ = 8.0 Hz, $J_{2f,3f}$ = 9.3 Hz, H-2f); HRMS found *m/z* 1013.3172 $[M+Na]^+$, calcd for $C_{36}H_{62}NaO_{31}$ 1013.3173; HRMS found *m/z* 1029.2920 $[M+K]^+$, calcd for $C_{36}H_{62}KO_{31}$ 1029.2912.

4.12. Biological tests

4.12.1. Evaluation of cytotoxicity

The cytotoxicity was studied on five distinct cell lines obtained from the european ECAC collection and on skin diploid fibroblasts which were provided by BIOPREDIC International Company. They

included two human colon carcinoma cells Caco2 and HCT 116 representative of two distinct differentiated and highly colon tumorigenic tumors, respectively, a differentiated highly growing HUH7 hepatocarcinoma cells, the NCI lung and PC3 prostate tumor cells. They were grown according to the providers recommendations.

The cytotoxicity assay was based on an automated imaging analysis as followed: 4×10^3 cells were seeded in 96-multiwell plates and led for 24 h for attachment, spreading and growth. Then, they were exposed for 24 and 48 h to increasing concentrations of the compounds, ranging from 0.1 to 25 μM in a final volume of 80 μL of culture medium. They were fixed with 4% paraformaldehyde solution and nuclei were stained with Hoechst 3342 and counted according to automated imaging quantification. Four pictures per well were obtained with a high speed camera and statistical analyses were established using the Simple PCI software. In addition, imaging analysis allowed detection of possible cell morphology changes.

4.12.2. Animals

Female, 6–8 weeks old, BALB/c mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

4.12.3. Phagocytosis of peripheral blood cells

Twenty four hours after ip injection with oligosaccharides, the mice were sacrificed. Peripheral blood from the orbital plexus was collected into heparin (5 IU/mL; Sigma St. Louis, MO). Phagocytosis of HEMA particles has been done as previously described.[17] Following incubation, two blood smears on slides were prepared from two parallel samples, stained with Accustain (Sigma) and evaluated. Differential counts and numbers of phagocytosis cells were established. Cells with at least three engulfed particles were considered positive.

4.12.4. Phagocytosis of peritoneal cells

Peritoneal macrophages were isolated from the peritoneal cavities of mice injected with oligosaccharides. The cells were diluted in RPMI-1640 medium with 5% fetal calf serum (Hyclone, Logan, UT) to 1×10^7 and incubated with HEMA particles as described earlier.[17] Phagocytosis and differential counts were evaluated as mentioned above.

4.12.5. Evaluation of IL-2 production

Purified spleen cells (2×10^6/mL in RPMI-1640 medium with 5% FCS) were added into wells of a 24-well tissue culture plate. After addition of 1 μg of Concanavalin A into positive-control wells, cells were incubated for 72 h. in a humidified incubator (37 °C, 5% CO_2). At the endpoint of incubation, supernatants were collected, filtered through 0.45 μm filters and tested for the presence of IL-2.[26] Levels of the IL-2 were measured using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN).

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Supplementary data

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