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Glucan-like synthetic oligosaccharides: iterative synthesis of linear oligo-β-(1,3)-glucans and immunostimulatory effects

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Small reducing and linear oligo-β-(1,3)-glucans, which are able to act as phytoallexin elicitors or as immunostimulating agents in anticancer therapy, were synthesized according to an iterative strategy that involved a unique key monosaccharidic donor. To avoid anomeric mixtures, the reducing entity of the target oligomers was first locked with benzyl alcohol and further selective deprotection of the 3-OH with DDQ afforded the desired building block as an acceptor. The latter was then used in a second cycle of glycosylation/deprotection to afford the desired disaccharide, and successive reiterations of this process provided the desired oligomers. Unusual conformational behaviors were observed by standard NMR sequences and supported by NOESY studies. Finally, removal of protecting groups afforded free tri-, tetra-, and pentaglucosides in good overall yields. Two oligosaccharides representing linear laminaritetraose and laminaripentaose were compared to the recently described β-(1,3)-glucan phycarine. Following an intraperitoneal injection, the influx of monocytes and granulocytes into the blood and macrophages into the peritoneal cavity was comparable to that caused by phycarine. Similarly, both oligosaccharides stimulated phagocytic activity of granulocytes and macrophages. Using ELISA, we also demonstrated a significant stimulation of secretion of IL-1β. Together these results suggest that the synthetic oligosaccharides have similar stimulatory effects as natural β-(1,3)-glucans.

Key words: carbohydrates/glycosides/glycosylation/immunostimulation/oligosaccharides

Introduction

β-(1,3)-Glucans were originally identified as essential constituents of the cell wall in fungi (Kobayashi *et al*., 1974) or as major storage source in brown seaweed (Black *et al*., 1951). These renewable polysaccharides are now classified as biological response modifiers. In this context, Albersheim has isolated and characterized a β-glucan fragment able to elicit the biosynthesis of phytoallexins, which are natural antibiotics in plants (Sharp *et al*., 1984a,b). Then, interest in poly-β-(1,3)-glucosides rapidly reached the human health field since immunostimulating and antitumoral properties were established (Vetvicka and Yvin, 2004; Bohn and BeMiller, 1995). In addition, numerous reports demonstrated that these polymers could also enhance the resistance of hematopoiesis in gamma-irradiated mice (Hofer and Popísil, 1997).

In connection with biological activities of β-(1,3)-glucans, Bohn and BeMiller (1995) published a survey devoted to knowledge accumulated until the mid-1990s about structure–functional activity relationships. They emphasized the importance of the β-(1,3)-glucan backbone but also many noticeable contradictory data that were previously published on the influence of molecular weight, water solubility, degree of 6-*O*-substitution by glucopyranosyl units, global chain conformation, and intermolecular associations (Falch *et al*., 2000) on antitumor activity and on mechanisms involved by these glucans used as biological response modifiers. Nevertheless, it is generally assumed that β-(1,3)-glucans are able to stimulate the nonspecific immune system against bacterial, viral, mycotic, and microparasitic infections as well as against malignant cell growth. On the other hand, current studies describing cellular recognition events finally elucidated the molecular role of these polysaccharides and especially their interactions with two specific binding sites, that is, the lectin domain of the complement receptor type 3 (CR3) (Ross *et al*., 1999; Xia *et al*., 1999; Yan *et al*., 1999) and the dectin-1 molecule, located on macrophages and white blood cells (Brown and Gordon, 2001). All these biological activities explain why purification (Lépagnol-Décamps *et al*., 1998) and structural elucidation of laminarins from various sources (Dong *et al*., 2002; Lowman *et al*., 2003; Read *et al*., 1996; Schmid *et al*., 2001), their chemical derivatization to improve their properties (de Nooy *et al*., 2000), and their enzymatic and/or chemical synthesis (Viladot *et al*., 1998) are still topics of significance. In addition, the biological variability among individual batches of natural glucans made the possibility of immunologically highly active oligosaccharides extremely important.

Among existing synthetic approaches, a number are connected with the preparation β-(1,6)-branched oligo-β-(1,3)-glucans (Amaya *et al*., 2001; He *et al*., 2002; Ning *et al*., 2002, 2003; Takeo and Tei, 1986; Takahashi *et al*., 2002; Tanaka *et al*., 2003; Zeng and Kong, 2003; Zeng *et al*., 2002; Zhao *et al*., 2003), but few methods were proposed for the synthesis of linear laminara-oligosaccharides. In fact, only unbranched methyl oligoglucosides were obtained (Takeo *et al*., 1993). In this context, and considering the

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newly discovered activities of small fragments of laminarins (Miyanishi *et al.*, 2003), we disclose herein with full details of synthesis and characterization an innovative route to linear and reducing oligo-β-(1,3)-glucans and their immunostimulatory effects, which were found to be as good as the natural polymers. Our synthetic methodology is based on an iterative two-step elongation of the glycosidic chain that involves only one monosaccharidic building block. The latter was designed so that it could successively act as a donor and as an acceptor. Reiteration of this glycosylation/deprotection strategy leads to laminaribi-, laminaritri-, laminaritetra-, and laminaripentaose **1**–**4**, respectively (Figure 1). We also described unusual conformational changes for several intermediates observed during this work.

β-(1,3)-Glucans have been extensively studied for their immunological and pharmacological effects. More than 900 papers describing the biological activities of β-(1,3)-glucans exist. Thus far, strong immunostimulating effects of β-(1,3)-glucans have been demonstrated in all tested animal species. However, just like any other natural product, various glucans cannot be 100% purely prepared, do not have a constant molecular weight, and suffer from significant batch-to-batch differences, all of which can affect some of their clinical effects. It is important, therefore, to evaluate the possibility of using synthetic oligosaccharides based on structure of β-(1,3)-glucans.

Results

The shorter route to laminaribiose **1** lies on direct glycosylation of the well-known diacetone glucose (**5**) (He *et al.*, 2002; Ning *et al.*, 2002; Zeng and Kong, 2003; Zeng *et al.*, 2002; Zhao *et al.*, 2003). Unfortunately, in our hands, this coupling using a peracylated thioglucosides or trichloroacetimidate gave the desired intermediate but invariably in admixture with a gentiobiose precursor, resulting from acid-catalyzed intramolecular transketalation and further 6-*O*-glycosylation, even with the more stable dicyclohexylidyl glucose as an acceptor. To avoid this side reaction, we reinvestigated our approach and assumed that the use of a unique intermediate, able to act alternatively as a donor for β-glucosidation and as a precursor of 3-OH acceptor, would gain in efficacy and so is highly desirable. This led us to choose the 2-naphthylmethyl (NAP) group (Borbás *et al.*, 2002; Gaunt *et al.*, 1998; Sarkar *et al.*, 1997, 2000; Xia *et al.*, 2000, 2001; Wright *et al.*, 2001) for an orthogonal protection of the 3-position on the basis of its higher stability under acidic conditions than that of its *p*-methoxybenzyl

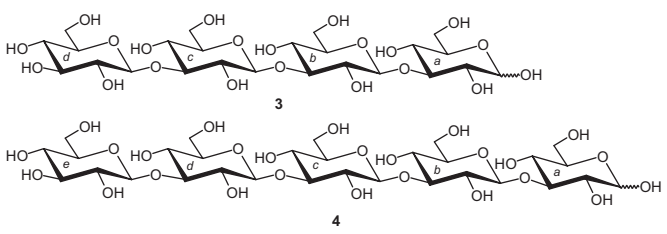
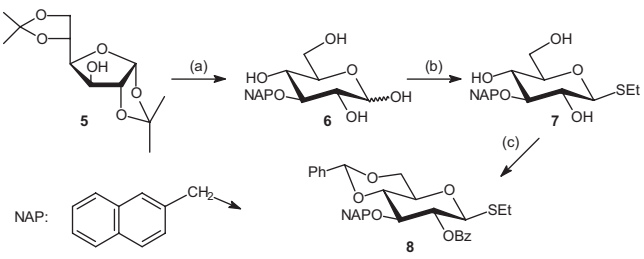


Fig. 1. Chemical structures of linear oligomers of β-(1'3)-glucans.

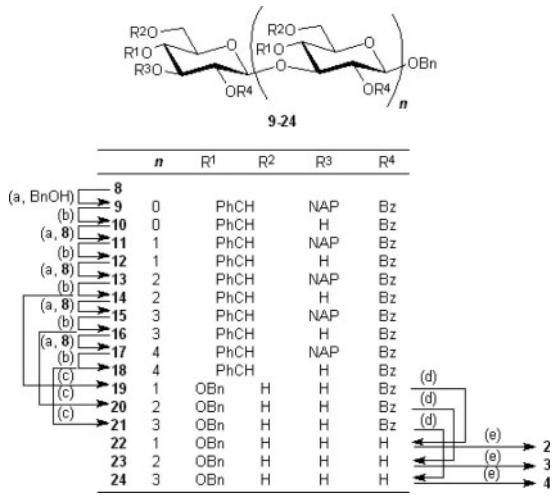


Conditions: (a) 1. NAPBr, NaH; 2. H_2O, THF, IR 120 (H^+-form) (80% for 2 steps); (b) 1. Ac_2O, AcONa; 2. EtSH, $BF_3.OEt_2$; 3. NaOMe, MeOH (67% for 3 steps); (c) 1. $PhCH(OMe)_2$, CSA; 2. BzCl, Pyr (84% for 2 steps).

Scheme I. Synthesis of the key building block **8**. Conditions: (**a**) 1. NAPBr, NaH; 2. H_2O, THF, IR 120 (H^+-form) (80% for 2 steps); (**b**) 1. Ac_2O, AcONa; 2. EtSH, $BF_3.OEt_2$; 3. NaOMe, MeOH (67% for 3 steps); (**c**) 1. $PhCH(OMe)_2$, camphorsulfonic acid (CSA); 2. BzCl, Pyr (84% for 2 steps).

counterpart. Therefore, the synthesis began with Williamson etherification of **5** followed by resin-assisted hydrolysis of ketal groups (Scheme I). The resulting product **6** gave the thioglucopyranoside **7** by running through three reactions (acetylation, Ferrier thioglycoside synthesis, and methanolysis) without intermediate chromatographic purification. Subsequent acidic transacetalization of benzaldehyde dimethylacetal followed by 2-*O*-benzoylation afforded the target compound **8**. This key building block was efficiently obtained on a 100-g scale in an average yield near 90% for each of the seven steps.

With this central product in hand, we could explore the elongation of the saccharidic chain. Practically, **8** reacted first with benzyl alcohol under activation by *N*-iodosuccinimide (NIS) and a catalytic amount of triethylsilyl trifluoromethanesulfonate (TESOTf), and afforded the required β-glucoside **9** in 89% yield (Scheme II). Further deprotection of **9** on oxidative cleavage of the 3-*O*-NAP group with 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ) in the presence of methanol (Borbás *et al.*, 2002; Xia *et al.*, 2000, 2001; Wright *et al.*, 2001) gave **10** in 85% yield without alteration of the 4,6-*O*-benzylidene ring. Subsequently, a second cycle of the iterative process starting from **8** as donor and **10** as the second acceptor provided successively **11** and **12** with a similar efficiency. Precursors of laminaritriose **13** and **14** were further prepared from **8** and the previously obtained disaccharide **12**. A fourth and a fifth cycle gave the tetra- and pentaglucosides **15**, **16** and **17**, **18**, respectively, in good overall yields. It is interesting to note that glycosylation of the tetrasaccharidic acceptor **16** was significantly improved by substituting TESOTf by tin(II) triflate (Gelin *et al.*, 2000). Finally, to improve these results, we also performed the iterative approach by substituting the naphthylmethyl group by the common allyl one. Although the synthesis of the corresponding key donor was quite efficient, troubles and lost of reproducibility were rapidly observed for both deprotection and glycosylation steps with tri- and higher oligoglucosides (Jamois, 2003). In fact, fine-tuning of reactivity between designed donor **8** and acceptors was best achieved using the NAP protection instead of another one.



Conditions : (a) NIS, TESOTf or $Sn(OTf)_2$ (**9**: 89%; **11**: 83%; **13**: 86%; **15**: 88%; **17**: 74%); (b) DDQ, CH_2Cl_2, MeOH (**10**: 85%; **12**: 79%; **14**: 78%; **16**:80%; **18**:86%) ; (c) H_2O, MeOH, CSA or PTSA, H_2O (**19**: 77%; **20**: 73%; **21**: 76%); (d) MeONa, MeOH (**22**: 100%; **23**: 99%; **24**: 99%); (e) H_2, $Pd(OAc)_2$, EtOH, H_2O (**2**: 95%; **3**: 96%; **4**: 94%).

Scheme II. Preparation of protected oligo-β-(1,3)-glucans **9–24** and free oligoglucosides **2–4**. Conditions: (**a**) NIS, TESOTf or $Sn(OTf)_2$ (**9**: 89%; **11**: 83%; **13**: 86%; **15**: 88%; **17**: 74%); (**b**) DDQ, CH_2Cl_2, MeOH (**10**: 85%; **12**: 79%; **14**: 78%; **16**:80%; **18**:86%) ; (c) H_2O, MeOH, CSA or p-Toluenesulfonic acid, H_2O (**19**: 77%; **20**: 73%; **21**: 76%); (**d**) MeONa, MeOH (**22**: 100%; **23**: 99%; **24**: 99%); (e) H_2, $Pd(OAc)_2$, EtOH, H_2O (**2**: 95%; **3**: 96%; **4**: 94%).

At this stage of our work, our attention was directed toward the characterization of these new synthons. However, within a molecule, only few structural differences really exist between the glucosyl residues and more particularly within the tetra- and the pentamers. This involved some uncertainty about assignment of nuclear magnetic resonance (NMR) signals. To meet this challenge, application of standard 2D NMR techniques (correlation spectroscopy [COSY] ^1H-^{13}C correlation) but also COSY long-range sequences that allowed connection between H-1 and other protons of the same entity were necessary. Moreover, the signal induced by the free 3-OH in **12**, **14**, and **16** was an excellent starting information to elucidate the spectra (Tables I and II).

Table I. Chemical shifts of anomeric centers for compounds **11–18**

	δ (ppm)				
Compound	C-1a	C-1b	C-1c	C-1d	C-1e
11	99.6	100.6	—	—	—
12	99.6	100.4	—	—	—
13	99.5	97.7	98.3	—	—
14	99.5	98.0	98.2	—	—
15	99.5	98.4	96.9	99.0	—
16	99.5	98.5	97.0	98.8	—
17	99.4	98.1[a]	97.2[a]	96.8[a]	98.6
18	99.1	98.1[b]	97.2[b]	96.8[b]	98.4[b]

[a,b]Signals may be interchanged.

As expected, the data first shown that C-1 chemical shifts and $^3J_{1,2}$ values observed for disaccharides **11** and **12** are relevant for two β-glucosidic linkages ($\delta_{C\text{-}1}$ ~ 99–100 ppm, $^3J_{1,2}$ ~ 7.5 Hz). However, from three to five glucopyranosyl residues, we were intrigued by significant modifications: (1) upfield chemical shifts of 1–2 ppm for all C-1 but C-1a, especially for anomeric center of the nonterminal entities; and (2) values of coupling constant of the middle units than that observed for the terminal ones ($\Delta J_{1,2}$ ~ –2 to –3 Hz, $\Delta J_{2,3}$ ~ –1 to –2 Hz, $\Delta J_{3,4}$ ~ –0.5 to –1 Hz). As a consequence, NMR data of compounds **11** and **12** seem to be quite acceptable for a β-D-Glc*p*-(1,3)-β-D-Glc*p* chain, whereas those compiled for the central core of tri-, tetra-, and pentasaccharides **13–18** could be in accordance with neither β-linkages nor α-connections or orthoesters as potential undesirable products. To explain these results, we first expected that desired β-glucosidic linkages were efficiently formed due to the benzoyl-directed protection and then assumed that the usual $^4C_1$ conformation of middle glucopyranosyl residues in **13–18** undergoes a strong ring distortion. Because NMR data of 3-*O*-etherified derivatives **13** and **15** present tight similarity with that of compounds **14** and **16** bearing a free 3-OH, this phenomenon cannot be connected with the 3-*O*-NAP protecting group. We thus hypothesized that important sterical effects are involved by benzylidene rings and that minimization of these contributions requires conformational adjustment for each nonterminal units. For further confirmation, nuclear Overhauser effect (NOE) spectroscopy (NOESY) experiments were carried out on the trisaccharidic derivative **14**. Besides anticipated NOE correlations between H-1c and H-3b, irradiation of H-1c and H-1a signals resulted in NOE enhancements of H-5c and H-5a, and to a lower extent, of H-3c and H-3a, respectively. Although irradiating H-1b involved positive NOE effects with H-3a, no other significant correlation could be established, neither with H-3b nor with H-5b. Consequently, these observations are indicative of a longer distance between H-1b and H-5b than that observed for the corresponding protons in *a* and *c* units. These results corroborate well the ring distortion observed only for the sugar ring located in the core of the trisaccharide **14** and are relevant to an average conformation on the pseudorotational itinerary between $^4C_1$ to $^{1,4}B$ or $B_{2,5}$ through $^4H_5$ and $^1S_5$ (Bentley, 1972).

To demonstrate the impact of acetal protecting groups on this spatial behavior, the benzylidene rings were first removed under acidic conditions and products **14**, **16**, and **18** gave the corresponding derivatives **19**, **20**, and **21**, respectively. As expected, these latter compounds were characterized by NMR data usually observed for β-glucopyranosides whatever the location of the glucosyl unit along the chain. Successive Zemplén transesterification and hydrogenolysis finally gave the required free tri-, tetra-, and pentasaccharides **2**, **3**, and **4**.

In conclusion, the key monoglucopyranosidic donor **8** was readily obtained in large scale. The efficiency and versatility of the designed iterative process thus demonstrated allowed the synthesis of structurally well-defined short, linear, reducing oligo-β-(1,3)-glucans and so represents an alternative to enzymatic hydrolysis of natural laminarins. More particularly, our study highlighted fine-tuning of reactivity between 3-*O*-NAP glucosyl donor and glucosidic acceptors so that assembly of linear oligoglucans up to the pentasaccharide level was attained. It was also interesting

Table II. ^{3}J coupling constants for compounds **11–16**

	J (Hz)															
Compound	1a,2a	1b,2b	1c,2c	1d,2d	2a,3a	2b,3b	2c,3c	2d,3d	3a,4a	3b,4b	3c,4c	3d,4d	4a,5a	4b,5b	4c,5c	4d,5d
11	7.6	7.2	—	—	9.0	7.7	—	—	9.1	9.2	—	—	9.5	9.6	—	—
12	7.5	7.3	—	—	8.2	9.1	—	—	9.0	9.8	—	—	9.5	9.6	—	—
13	7.9	4.3	7.6	—	8.9	4.1	8.0	—	9.3	8.1	9.8	—	9.6	9.0	9.5	—
14	7.9	4.3	7.5	—	8.8	4.0	8.6	—	9.2	8.5	9.3	—	9.7	9.0	9.4	—
15	7.8	3.1	5.4	7.4	8.6	ND	5.3	7.9	9.0	ND	8.8	9.2	9.2	ND	8.8	8.7
16	7.9	5.6	5.3	7.6	9.2	5.2	5.1	8.7	9.0	8.5	8.5	9.2	9.2	ND	9.1	9.4

ND: not determined.

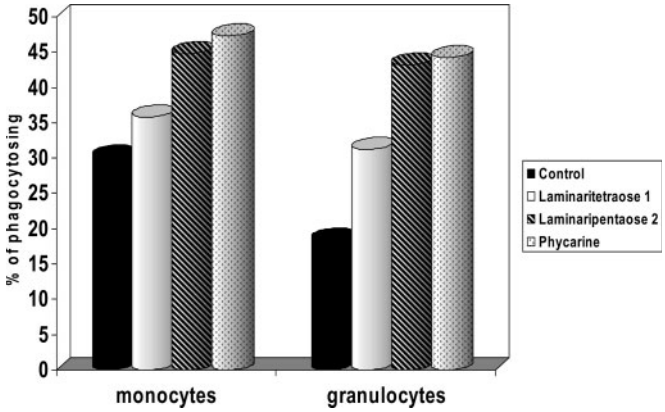


Fig. 2. Potentiation of phagocytosis of synthetic microspheres (HEMA particles) by IP-injected phycarine or two oligosaccharides. Monocytes and granulocytes with three or more HEMA particles were considered positive.

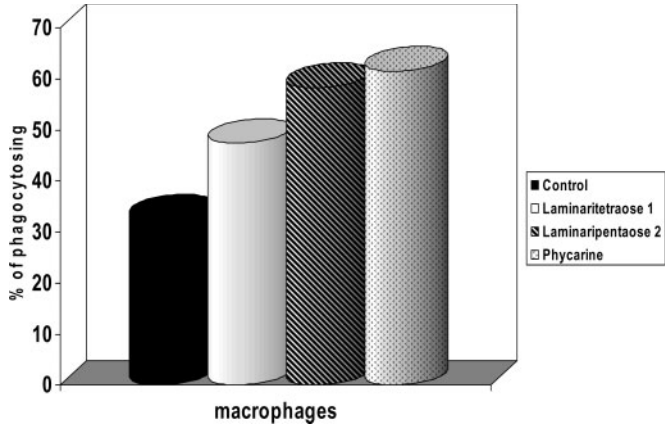


Fig. 3. Potentiation of phagocytosis of synthetic microspheres (HEMA particles) by IP-injected phycarine or two oligosaccharides. Peritoneal macrophages with three or more HEMA particles were considered positive.

to note that the protected intermediates underwent unexpected conformational changes for each nonterminal residues lying on steric hindrance involved by benzylidene rings. This effect had however no consequence on further glycosylation steps.

The effects of various glucans on macrophages are well established. However, to demonstrate that synthetic oligosaccharides really exhibit an immunomodulatory characteristics, an evaluation of phagocytosis is necessary. We measured the effects of different doses of phycarine on phagocytosis of synthetic 2-hydroxyethyl methacrylate (HEMA) microspheres in peripheral blood (Figure 2) and peritoneal cavity (Figure 3). The internalization of synthetic particles was notably more comparable to phycarine application, and at higher doses (250 μg/mouse) caused significant elevation even 4 days after injection (data not shown). We also measured the effects of administration of these two oligosaccharides on cellularity in both peripheral blood and peritoneum. Data summarized in Figures 4 and 5 show a significant increase in the number of neutrophils and peritoneal macrophages.

We also examined the effect of a single intraperitoneal injection of oligosaccharides on systemic in vivo release of IL-1β. Peripheral blood was isolated at three different intervals after the injection and the obtained serum was stored at –80°C for no more than 1 week. The data summarized in Figure 6 show significant elevation in levels of IL-1β at

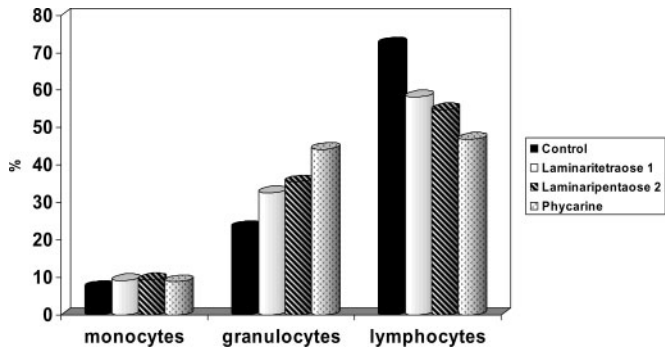


Fig. 4. Effect of IP injecti on of 100 μg phycarine or oligosaccharides on differential counts in peripheral blood.

every tested interval, the level of secretion was highest in case of phycarine.

In the final phase of the series of experiments, mice challenged withPtas64 mammary tumors were tested for a therapeutic response to daily intraperitoneal injections of either phycarine or individual oligosaccharides (Figure 7). This experiment was repeated three times with similar results. To evaluate the possible effects of lipopolysaccharide contamination, we also used lipopolysaccharide-free

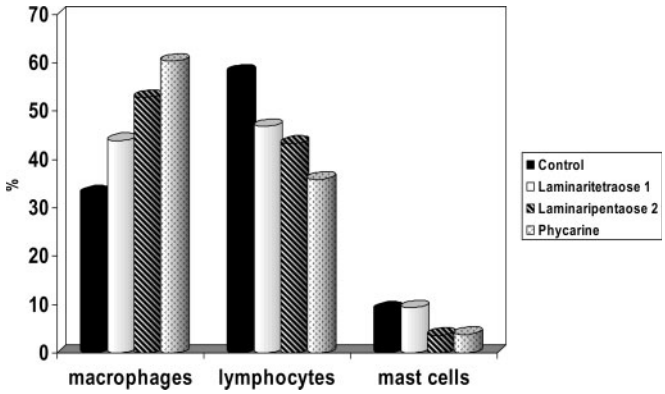


Fig. 5. Effect of IP injection of 100 μg phycarine or oligosaccharides on differential counts in peritoneal cavity.

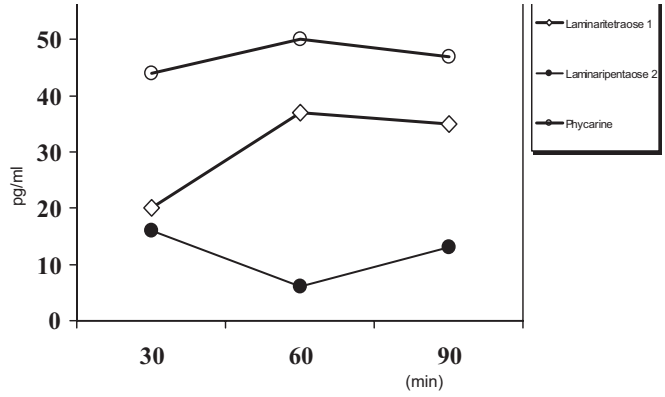


Fig. 6. Effect of IP injection of 100 μg phycarine or oligosaccharides on levels of IL-1β in peripheral blood.

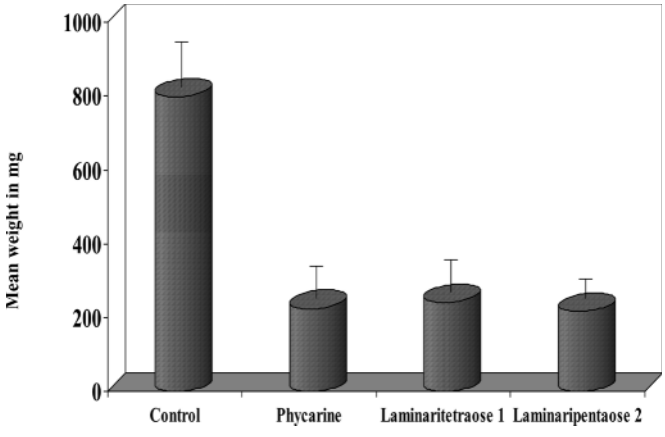


Fig. 7. Phycarine or oligosaccharide therapy of Balb/c mice with Ptas64 mammary carcinoma. Data from three experiments are shown. After 2 weeks of therapy, and for each experiment, four groups of mice were tested for a response for phycarine or oligosaccharides by weighing the tumors. Additionally, individual groups were given daily IP injections of 100 μg of tested substance, respectively. The control group of mice received daily IP PBS. Each value represents the mean ± SD.

phycarine with identical results (data not shown). This data showed the strong inhibition of tumor growth by both phycarine and oligosaccharides.

Discussion

Branched and linear β-(1,3)-glucans originated from numerous sources, such as mushrooms, yeast, or seaweed and are well known biological response modifiers. However, their rate of success is often varying and unpredictable (Kimura *et al*., 1994; Tanabe *et al*., 1990). This can be at least partly ascribed to the differences between individual batches and impurities due to the imperfect isolation processes. The possibility of using higly active biological synthetic oligosaccharides, based on know glucan structure and compared to some kind of benchmark biological activities, is therefore important. The pharmacokinetic parameters of β-(1,3)-glucans are extremely important for calculating pharmaceutical dose levels. Although large glucans appear to be better because of their resistance to glomerular filtration and saturation of liver clearance, glucans that are too large have a higher potential for producing undesirable side effects.

Without any doubts, some commercially available oligosaccharides corresponding to our oligosaccharides exist on the market. However, our evaluation of the commercial preparations revealed presence of significant levels of impurities (e.g., hexasaccharides in samples of pentasaccharides or presence of laminaripentaoses in samples of laminarintetraoses), mostly due to the use of enzymatic hydrolysis of higher polymers. This mixture of oligosaccharides is virtually impossible to separate completely. On the other hand, no traces of other oligosaccharides were detected in our samples when tested by mass spectroscopy. Therefore only our synthetic oligosaccharides have been used throughout the present study.

For comparison of immunostimulating properties, we used phycarine, a recently described seaweed-derived glucan (Vetvicka and Yvin, 2004). Structural analysis showed that phycarine (laminarin) is an essentially linear glucan composed of ~33 glucopyranose units joined by acetalic β-(1,3) linkages with molecular weight of 5000 Da with purity higher than 97%.

Glucans are well known to stimulate phagocytosis (Abel *et al*., 1989; Yan *et al*., 2003). Therefore, one of the first tests of the immunological characteristics is the phagocytosis. We used synthetic microspheres based on HEMA. These HEMA particles, in contrast to yeast particles, have a slight negative charge and therefore do not specifically adhere to the cell surface, which guarantees that only actively phagocytosing cells will internalize these inert particles (for review see Vetvicka and Formusek, 1987). In both peritoneal macrophages and peripheral blood cells, we found significant stimulation of phagocytosis at the level comparable with phycarine. In addition, the influx of macrophages into the peritoneal cavity lasted at least 3 days (data not shown), which further shows the activation of macrophages by these oligosaccharides.

It is hypothesized that the immunostimulating actions of β-glucans are, at least in part, caused by potentiation of a synthesis and release of several cytokines such as TNFα, IFNγ, and IL-1β (Ohmura *et al*., 2001; Seljelid *et al*., 1989). This cytokine-stimulating activity is thought to be dependent

on the triple helix conformation (Falch *et al.*, 2000). Most glucans, including oligomer prepared from laminarin (Miyanishi *et al.*, 2003), have been shown to stimulate TNFα both in vivo and in vitro (Ross *et al.*, 1999). The stimulation of TNFα was often found to be secondary effect of a massive release of IL-1 (Ohmura *et al.*, 2001). Therefore we compared the levels of IL-1 after injection of these synthetic oligosaccharides and phycarine. The data obtained not only showed similar stimulation but also correlated with findings of effects of lentinan in treatment of human cancer (Ross *et al.*, 1999) and stimulation caused by paramylon (Kondo *et al.*, 1992). Our data suggest that the triple helix conformation generally observed for glucans with degree of polymerization near 30 is not necessary for tetra- and penta-β-(1,3)-glucans to obtain immunostimulating effects. The biological effect of these oligosaccharides was further evaluated by measuring the inhibition of breast cancer growth as determined by the weight of the tumor. This was done after 2 weeks of therapy with either oligosaccharides or phycarine. In every case there was a significant inhibition of cancer growth.

Only very limited knowledge of glucan-based oligosaccharides and their immunological properties exist. Thus far, only laminarin-based oligomer (Miyanishi *et al.*, 2003) and β- and α-(1,3)-glucohexaoses were described as having immunostimulating characteristics comparable to parental β-glucan. The current spike of interest in commercial use of β-glucans clearly shifts the attention on the prospect of 99% pure synthetic oligomers without any contaminating biological impurities.

Material and methods

General methods

All reactions were performed under nitrogen. Thin-layer chromatography (TLC) analyses were carried out on precoated nonactivated plates (Merck 60 F_{254}; Darmstadt, Germany) with detection by UV absorption (254 nm) when applicable and charring with 5% H_2SO_4 in EtOH. For column chromatography, Merck 60H (5–40 μm) silica gel was used. Optical rotations were determined with a Perkin-Elmer 341 polarimeter at 20°C using a 1-dm cell. ^{1}H, ^{13}C, NMR spectra, and 2D COSY, heteronuclear multiple band correlation and NOESY experiments were recorded on a Brüker ARX 400 spectrometer; chemical shifts (δ) are given in ppm. Microanalyses and recording of mass spectra were performed by the Centre Régional de Mesures Physiques de l'Ouest (CRMPO, University of Rennes 1, France).

*Compound **6***

For compound **6** (3-*O*-NAP-D-glucopyranose), a 60% suspension of NaH in oil (21.7 g, 543 mmol) was added at 0°C to a solution of **5** (117.7 g, 452 mmol) and NAP bromide (100 g, 452 mmol) in dry N,N-Dimethylformamide. The mixture was allowed to reach room temperature and stirred for 3 h. The excess of NaH was then neutralized with MeOH and the product crystallized out by adding iced water (2 L) under vigorous agitation. The supernatant was removed and the solid taken up into CH_2Cl_2. After decanting, the organic layer was dried ($MgSO_4$) and concentrated.

The product thus obtained was dissolved in acetone (270 ml) and water (270 L) and resin IR 120 (H^+ form) (420 g) were successively added. The reaction media was heated at 60°C for 2 days. The resin was filtered off and washed with MeOH, and the filtrate was neutralized by adding few drops of an aqueous solution of sodium bicarbonate. After removal of the solvents, the resulting solid was washed with toluene to give **2** (116 g, 80%): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.5; ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.78–7.31 (m, NAP), 4.97 [d, ^2J 11.3 Hz, 1 H, CH_2(NAP)β], 4.93 [d, 1 H, CH_2(NAP)β] and Table III; ^{13}C-NMR (CD_3OD, 100 MHz): δ 138.0, 134.8, 134.4 [C-*q* (NAP)], 128.9, 128.8, 128.6, 127.5, 127.4, 127.3, 126.9, 126.8 (NAP), 76.3 [CH_2(NAP)α[a]], 73.0 [CH_2(NAP)β] and Table IV; (a): value can be inverted with those listed in Table IV; electrospray ionization mass spectrometry (ESI-MS): ([M+Na]$^+$, $C_{17}H_{20}O_6Na$): *m/z* calculatedd 343.1158, found 343.1159.

*Compound **7***

For compound **7** (Ethyl 3-*O*-NAP-1-thio-β-D-glucopyranoside), compound **6** (116 g, 362 mmol), sodium acetate (59.4 g, 724 mmol) and acetic anhydride (680 ml, 7.24 mol) were successively introduced in a flask. The mixture was refluxed for 2 h and then poured onto water (5 L). After stirring overnight, the solid was filtered off, washed with a 5% aqueous solution of sodium bicarbonate, filtered off again, washed with water, and dried ($MgSO_4$).

To the resulting product dissolved in CH_2Cl_2 (885 ml) were successively added at 0°C ethanethiol (20.5 ml, 399 mmol)

Table III. ^{1}H-NMR chemical shifts and ^3J coupling constants for monosaccharidic compounds **6–10**

	δ (ppm) (*J*, Hz)						
Compound	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6 ($J_{6,6'}$)	H-6' ($J_{5,6'}$)
6β	4.42 (7.8)	3.18–3.24 (8.8)	3.32 (9.0)	3.38 (9.1)	3.18–3.24 (2.4)	3.77 (11.9)	3.57 (5.9)
6α	5.02 (3.6)	ND	ND	ND	ND	ND	ND
7	4.36 (9.6)	3.53 (8.8)	3.46 (8.9)	3.62 (9.2)	3.37 (3.4)	3.87 (12.0)	3.75 (4.9)
8	4.59 (10.0)	5.36 (8.9)	3.93 (9.0)	3.88 (9.2)	3.56 (5.0)	4.41 (10.4)	3.84 (10.1)
9	4.58 (7.8)	5.40 (8.4)	3.84 (9.1)	3.90 (9.2)	3.45 (5.0)	4.40 (10.4)	3.86 (10.0)
10	4.70 (7.9)	5.26 (9.2)	3.99 (9.3)	3.68 (9.6)	3.49 (5.1)	4.42 (10.4)	3.85 (10.0)

ND: not determined.

Table IV. ^{13}C-NMR chemical shifts for monosaccharidic compounds **6–10**

	δ (ppm)					
Compound	C-1	C-2	C-3	C-4	C-5	C-6
6β	98.3	76.5[a]	86.4	71.6	78.0[a]	62.8
6α	94.2	71.7[b]	83.6	73.1[b]	74.0[b]	62.7
7	86.7	73.2	85.0	70.1	79.5	62.7
8	84.4	71.9	79.0	81.8	70.8	68.7
9	100.0	73.3	72.6	81.8	66.3	68.8
10	99.8	74.8	72.4	80.9	66.3	68.7

[a,b]Values can be inverted among themselves or with CH_2(NAP).

and boron trifluoride etherate (50.1 ml, 3.99 mmol). After 2 h stirring at 0°C, the organic solution was washed with a 5% aqueous solution of sodium bicarbonate abd water, then dried ($MgSO_4$) and concentrated.

To a methanolic solution of sodium methanolate, prepared from MeOH (200 ml) and sodium (703 mg, 306 mmol), was added a solution of the previously obtained intermediate in toluene (100 ml). After 4 h at room temperature, the reaction was quenched with IR 120 (H^+ form) resin, filtered, and concentrated. The resulting crude oil was then poured onto heptane (1 L). After filtration, the resulting solid was taken up with CH_2Cl_2 (600 ml), and the by-products were removed by washings with water (600 ml) at 40°C. The organic layer was recovered, dried, and evaporated to give **7** (88.3 g, 67% overall yield): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.5. mp: 78–80°C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.86–7.82 (m, 4 H, NAP), 7.51–7.47 (m, 3 H, NAP), 5.17 [d, 1 H, ^2J 11.8 Hz, CH_2(NAP)], 4.94 [d, 1 H, CH_2(NAP)], 2.73 (qd, 2 H, ^2J 7.4 Hz, ^3J 7.5 Hz, SCH_2CH$_3$), 2.68 (sl, 1 H, OH), 2.56 (s, 1 H, OH), 2.22 (sl, 1 H, OH), 1.33 (t, 3 H, SCH$_2$CH_3) and Table III; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 135.8, 133.4, 133.1 [C-*q* (NAP)], 128.6, 128.0, 127.8, 127.0, 126.3, 126.2, 125.9 (NAP), 74.9 [CH_2(NAP)], 24.7 (SCH_2CH$_3$), 15.5 (SCH$_2$$CH_3$) and Table IV; ESI-MS: ([M+Na]$^+$, $C_{19}H_{24}O_5SNa$): *m/z* calculated 387.1242, found 387.1244; ([M+K]$^+$, $C_{19}H_{24}O_5SK$): *m/z* calculated 403.0982, found 403.0997.

*Compound **8***

To a solution of compound **7** (50 g, 137 mmol) in acetonitrile (300 ml) were successively added benzaldehyde dimethylacetal (31 ml, 206 mmol) and anhydrous camphorsulfonic acid (6.4 g, 27 mmol). The mixture was heated at 55°C for 2 h, cooled to room temperature, and neutralized with triethylamine. After concentration, the residue was dissolved in CH_2Cl_2 and poured onto MeOH (1 L). After 1 night at 4°C, the intermediate was filtered off and washed with cold MeOH.

The crude product was further submitted to benzoylation under standard conditions using pyridine (300 ml) and benzoyl chloride (37 ml, 321 mmol). After stirring overnight at room temperature, the reaction media was poured onto MeOH (1 L). The resulting solid was filtered off, washed with MeOH, and dried to afford **8** (ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-NAP-1-thio-β-D-glucopyranoside) (50 g, 84% overall yield): TLC (toluene/EtOAc, 9:1): R_f = 0.6; mp: 137–139°C; $[\alpha]^{20}_D$ + 17 (*c* 1.0; CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.95–7.92 (m, 2 H, H arom.), 7.69–7.36 (m, 12 H, H arom.), 7.22 (dd, *J* 1.4 Hz, *J* 8.4 Hz, 1 H, H arom.), 5.64 (s, 1 H, C*H*Ph), 4.98 [d, ^2J 12.2 Hz, 1 H, CH_2(NAP)], 4.86 (d, 1 H, CH_2(NAP)], 2.77–2.64 (m, 2 H, SCH_2CH$_3$), 1.21 (t, *J* 7.4 Hz, 3 H, SCH$_2$CH_3) and Table III; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.3 (C = O), 137.3, 135.3, 133.3, 133.1, 133.0 (C-*q* arom.), 130.0, 129.2, 128.4, 128.1, 127.9, 127.7, 127.1, 126.3, 126.1, 126.0, 125.8 (C arom.), 101.4 (*C*HPh), 74.3 [CH_2(NAP)], 24.1 (SCH_2CH$_3$), 14.9(SCH$_2$$CH_3$), and Table IV; ESI-MS: ([M+Na]$^+$, $C_{33}H_{32}O_6SNa$): *m/z* calculated 579.1817, found 579.1820; ([M+K]$^+$, $C_{33}H_{32}O_6SK$): *m/z* calculated 595.1557, found 595.1559; ([M+Na+CH$_3$OH]$^+$, $C_{34}H_{36}O_7SNa$): *m/z* calculated 611.2080, found 611.2066. Analytical calculated for $C_{33}H_{32}O_6S$ (556.18): C, 71.20; H, 5.79; S, 5.76; found: C, 71.70; H, 5.74; S, 6.12.

Elongation chain: general procedure

Glycosidic coupling. NIS (1.1 or 1.2 equiv.) and a Lewis acid [0.1 eq, either TMSOTf, TESOTf, or Sn(OTf)$_2$] were successively added to a solution of donor **8** (1.1 equiv.) and glycosyl acceptor (1 equiv.) in the presence of 4-Å molecular sieves under nitrogen. The mixture was stirred at 0°C and, when TLC monitoring indicated completion of the reaction, quenched by addition of triethylamine. After filtration through a bed of celite, the solution was diluted with CH_2Cl_2, washed with a 10% aqueous solution of sodium thiosulfate and with water, dried ($MgSO_4$), and concentrated. The expected product was finally purified by flash chromatography.

Deprotection of NAP group. After dissolution of the resulting coupling compound (1 equiv.) in CH_2Cl_2/MeOH (4:1, v/v), DDQ (3 equiv.) was added and the mixture then stirred at room temperature. After completion of the reaction, the media was diluted with CH_2Cl_2, washed with a 5% aqueous solution of sodium bicarbonate, and washed again with water. The organic layer was dried ($MgSO_4$) and concentrated, and the pure target product was obtained after purification over silica gel.

*Compound **9***

This compound was obtained as described in procedure A, starting from **8** (312 mg, 0.56 mmol) in CH_2Cl_2 (2 ml) containing molecular sieves (100 mg), using benzyl alcohol (69.6 µl, 0.67 mmol) as an acceptor and NIS (139 mg, 0.62 mmol) and TESOTf (12.7 µl, 0.06 mmol) as a promotor. After 1 h stirring, work-up and chromatography (toluene/EtOAc, 17:3) enable collecting **9** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-NAP-β-D-glucopyranoside) (300 mg, 89%): TLC (toluene/EtOAc, 9:1): R_f = 0.6; mp: 142–144°C; $[\alpha]^{20}_D$ – 6 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δδ 7.89–7.86 (m, 2 H, H arom.), 7.67–7.07 (m, 17 H, H arom.), 5.62 (s, 1 H, C*H*Ph), 4.95 [d, ^2J 12.4 Hz, 1 H, CH_2(NAP)], 4.84 [d, 2 H, CH_2Ph, CH_2(NAP)], 4.58 (d, ^2J 13.6 Hz, 1 H, CH_2Ph) and Table III; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.1 (C = O), 137.3, 136.8, 135.3, 133.1, 133.0, 132.9 (C-*q* arom.), 129.9, 129.8, 129.1, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8,

127.7, 127.6, 127.0, 126.2, 126.1, 125.9, 125.8 (C arom.), 101.4 (C*H*Ph), 74.0 [*C*H_2(NAP)], 70.5 (*C*H_2Ph) and Table IV; ESI-MS: ($[M+Na]^+$, $C_{38}H_{34}O_7Na$): *m/z* calculated 625.2202, found 625.2194; ($[M+K]^+$, $C_{38}H_{34}O_7K$): *m/z* calculated 641.1942, found 641.1949; ($[M+Na+CH_3OH]^+$, $C_{39}H_{38}O_8Na$): *m/z* calculated 657.2464, found 657.2438.

*Compound **10***

For compound 10 (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside), the deprotection reaction was performed according to procedure B starting from **8** (420 mg, 0.70 mmol) in solvent (8.5 ml) and using DDQ (475 mg, 2.09 mmol). After 5.5 h stirring, work-up and chromatography (toluene/EtOAc, 9:1) gave **9** (274 mg, 85%): TLC (toluene/EtOAc, 9:1): R_f = 0.3; mp: 147–149°C; $[\alpha]^{20}_D$ – 84 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.03–8.00 (m, 2 H, H arom.), 7.62–7.58 (m, 1 H, H arom.), 7.52–7.43 (m, 4 H, H arom.), 7.41–7.36 (m, 3 H, H arom.), 7.23–7.19 (m, 5 H, H arom.), 5.57 (s, 1 H, C*H*Ph), 4.89 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.66 (d, 1 H, CH_2Ph), 2.84 (s, 1 H, OH-3) and Table III; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 166.0 (C = O), 137.0, 136.7, 133.4 (C-*q* arom.), 130.1, 129.6, 129.4, 128.4, 127.9, 127.8, 126.4 (C arom.), 102.0 (*C*HPh), 70.6 (*C*H_2Ph) and Table IV; ESI-MS: ($[M+Na]^+$, $C_{27}H_{26}O_7Na$): *m/z* calculated 485.1576, found 485.1574; ($[M+K]^+$, $C_{27}H_{26}O_7K$): *m/z* calculated 501.1316, found 501.1312; ($[M+Na+CH_3OH]^+$, $C_{28}H_{30}O_8Na$): *m/z* calculated 517.1838, found 517.1832.

*Compound **11***

Disaccharide **11** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-NAP-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) was synthesized according to procedure A, using **8** (802 mg, 1.44 mmol) and **10** (606 mg, 1.31 mmol) in CH_2Cl_2 (7.0 ml) containing molecular sieves (200 mg), NIS (324 mg, 1.44 mmol), and TESOTf (29.6 µl, 0.13 mmol). After 50 min at room temperature, work-up and chromatography (toluene/EtOAc, 19:1) afforded **11** (1.036 g, 83%): TLC (toluene/EtOAc, 4:1): R_f = 0.6; mp: 202–204°C; $[\alpha]^{20}_D$ – 1 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.74–7.02 (m, 32 H, H arom.), 5.53 (s, 1 H, C*H*Ph), 5.34 (s, 1 H, C*H*Ph), 4.84 [d, ^2J 12.4 Hz, 1 H, CH_2(NAP)], 4.74 (d, 1 H, CH_2(NAP)], 4.74 (d, ^2J 12.2 Hz, 1 H, CH_2Ph), 4.50 (d, 1 H, CH_2Ph), and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 164.7, 164.6 (C = O), 137.3, 137.2, 136.7, 135.3, 133.0, 132.9, 132.8, 132.7 (C-*q* arom.), 129.8–125.7 (C arom.), 101.5, 101.1 (*C*HPh), 73.5[a] [*C*H_2(NAP)], 70.3 (*C*H_2Ph) and Table VI; (a): value can be inverted with those listed in Table VI. Analyitcal calculated for $C_{28}H_{38}O_{19}$: C, 72.79; H, 5.48; found: C, 73.12; H, 5.48.

Table V. ^{1}H-NMR chemical shifts and ^3J coupling constants for disaccharides **11** and **12**; trisaccharides **13**, **14**, and **19**; and tetrasaccharidic derivatives **15** and **16**

		δ (ppm) (^3J, Hz)						
Product		H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$)	H-6 ($J_{6,6'}$)	H-6' ($J_{5,6'}$)
11	*Unit a*	4.56 (7.6)	5.31 (9.0)	4.11 (9.1)	3.84 (9.5)	3.49 (4.9)	4.36 (10.4)	3.80 (10.0)
	Unit b	4.85 (7.2)	5.28 (7.7)	3.74 (9.2)	3.90 (9.6)	3.37 (4.9)	4.16 (10.4)	3.68 (10.0)
12	*Unit a*	4.52 (7.5)	5.25 (8.2)	4.08 (9.0)	3.77 (9.5)	3.44 (4.8)	4.30 (10.4)	3.73 (10.0)
	Unit b	4.83 (7.3)	5.03 (9.1)	3.76 (9.8)	3.57 (9.6)	3.28 (4.9)	4.10 (10.4)	3.60 (10.0)
13	*Unit a*	4.45 (7.9)	4.86 (8.9)	4.07 (9.3)	3.18 (9.6)	3.37 (4.9)	4.31 (10.3)	3.64 (10.0)
	Unit b	4.86 (4.3)	5.08 (4.1)	3.97 (8.1)	4.07 (9.0)	3.55 (3.9)	4.11 (9.1)	3.52 (9.7)
	Unit c	5.06 (7.6)	5.34 (8.0)	3.85 (9.8)	3.91 (9.5)	3.50 (4.4)	4.22 (10.4)	3.72 (10.1)
14	*Unit a*	4.47 (7.9)	4.95 (8.8)	4.09 (9.2)	3.21 (9.7)	3.38 (4.9)	4.31 (10.5)	3.65 (10.3)
	Unit b	4.90 (4.3)	5.13 (4.0)	4.01 (8.5)	4.07 (9.0)	3.56 (3.7)	4.12 (10.4)	3.47 (9.7)
	Unit c	5.11 (7.5)	5.20 (8.6)	3.96 (9.3)	3.67 (9.4)	3.47 (4.8)	4.22 (10.3)	3.69 (10.2)
19	*Unit a*	4.41 (7.9)	4.85 (9.4)	3.75–4.86 (ND)	3.34–3.42 (ND)	3.34–3.42 (ND)	3.75–4.86 (ND)	3.53–3.64 (ND)
	Unit b	4.61 (8.0)	4.82 (9.5)	3.75–4.86 (ND)	3.34–3.42 (9.8)	3.27 (2.1)	3.75–4.86 (ND)	3.53–3.64 (5.7)
	Unit c	4.52 (8.1)	4.75 (9.0)	3.15–3.22 (ND)	3.15–3.22 (ND)	3.15–3.22 (ND)	3.75–4.86 (ND)	3.53–3.64 (ND)
15	*Unit a*	4.47 (7.8)	4.98 (8.6)	4.04 (9.0)	3.35 (9.2)	3.37–3.59 (4.3)	4.34[a] (10.3)	3.68–3.75[b] (ND)
	Unit b	4.75 (3.1)	4.75–4.77 (ND)	3.89–3.92 (ND)	3.37–3.59 (ND)	3.37–3.59 (ND)	4.09–4.12[a] (ND)	3.68–3.75[b] (ND)
	Unit c	4.95 (5.4)	5.12 (5.3)	4.00–4.03 (8.8)	3.95 (8.8)	3.37–3.59 (ND)	4.09–4.12[a] (ND)	3.37–3.59[b] (ND)
	Unit d	4.98 (7.4)	5.33 (7.9)	3.82 (9.2)	3.87 (8.7)	3.37–3.59 (4.9)	4.19[a] (10.4)	3.37–3.59[b] (ND)
16	*Unit a*	4.47 (7.9)	4.99 (9.2)	4.04 (9.0)	3.35 (9.2)	3.37–3.47 (4.6)	4.34[c] (10.4)	3.72[d] (9.9)
	Unit b	4.76 (5.6)	5.15 (5.2)	3.91 (8.5)	3.37–3.47 (ND)	3.37–3.47 (ND)	4.09–4.12[c] (ND)	3.67[d] (10.1)
	Unit c	4.97 (5.3)	4.82 (5.1)	4.05 (8.5)	3.95 (9.1)	3.54–3.58 (ND)	4.09–4.12[c] (ND)	3.37–3.47[d] (ND)
	Unit d	5.02 (7.6)	5.16 (8.7)	3.89 (9.2)	3.60 (9.4)	3.37–3.47 (4.9)	4.18[c] (10.4)	3.51[d] (10.0)

[a,b,c,d]Signals may be interchanged.
ND: not determined.

Table VI. ^{13}C-NMR chemical shifts for disaccharides **11** and **12**; trisaccharrides **13**, **14**, and **19**; and tetrasaccharidic derivatives **15** and **16**

	δ (ppm)																							
	Unit a						*Unit b*						*Unit c*						*Unit d*					
Product	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-3	C-4	C-5	C-6
11	99.6	73.5[a]	77.8	79.3	66.5	68.7[b]	100.6	73.7	78.2	80.9	66.0	68.8[b]	—	—	—	—	—	—	—	—	—	—	—	—
12	99.6	73.5	78.0	79.3	66.5	68.8	100.4	75.3	72.6	80.5	66.1	68.7	—	—	—	—	—	—	—	—	—	—	—	—
13	99.5	74.3	74.1	78.7	66.5	68.7	97.9	72.6	76.2	77.6	65.4	68.8	98.3	73.3	78.2	81.4	66.1	68.8	—	—	—	—	—	—
14	99.5	74.3	74.4	78.7	66.5	68.7	98.0	72.7	76.3	77.6	65.4	68.8	98.2	74.7	72.5	80.9	66.1	68.7	—	—	—	—	—	—
19	100.9	74.7	82.7	69.9[d]	77.8[e]	62.4[f]	102.3	74.4	83.9	70.1[d]	77.9[e]	62.4[f]	101.9	75.4	76.1	71.5	78.3	62.2[f]	—	—	—	—	—	—
15	99.5	74.0	75.1	78.8	65.5[a]	68.7[b]	98.4	73.5	74.2	77.4	65.5[a]	68.9[b]	99.6	72.5	76.9	78.2[c]	66.1[a]	68.7[b]	99.0	73.4	78.3[c]	81.3	66.4[a]	68.7[b]
16	99.5	73.9	75.1	78.8	65.5[d]	68.6	98.5	73.5	74.3	77.4	65.5[d]	68.6	97.0	72.5	76.8	78.2	66.0[d]	68.6	98.8	74.8	72.4	80.7	66.4[d]	68.6

[a,b,c,d,e,f]Signals may be interchanged

Compound ***12***

According to procedure B, compound **11** (217 mg, 0.23 mmol) and DDQ (154 mg, 0.68 mmol) were stirred for 4.5 h in solvent (4.3 ml) and further work-up and chromatography (toluene/EtOAc, 9:1) provided **12** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) (146 mg, 79%): TLC (toluene/EtOAc, 4:1): R_f = 0.4; mp: 219–221°C; $[\alpha]^{20}_D$ – 46 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.70–6.96 (m, 25 H, H arom.), 5.47 (s, 1 H, C*H*Ph), 5.27 (s, 1 H, C*H*Ph), 4.69 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.44 (d, 1 H, CH_2Ph), 2.55 (s, 1 H, OH-3b) and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.6, 164.7 (C = O), 137.2, 137.0, 136.6, 133.1, 133.0 (C-*q* arom.), 129.8–126.2 (C arom.), 101.7 (*C*HPh), 101.5 (*C*HPh), 70.3 (*C*H_2Ph) and Table VI. Analytical calculatd for $C_{47}H_{44}O_{13}$: C, 69.11, H, 5.43; found: C, 68.73, H, 5.51.

Compound ***13***

Compound **13** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-NAP-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) was prepared as described in procedure A, starting from **8** (503 mg, 0.90 mmol) in CH_2Cl_2 (10 ml), containing molecular sieves (1.0 g), and using disaccharide **12** (670 mg, 0.82 mmol), NIS (203 mg, 0.90 mmol), and TESOTf (19 µl, 0.09 mmol). After 50 min stirring, work-up and chromatography (toluene/EtOAc, 19:1.9:1) enabled collecting **13** (928 mg, 86%); TLC (toluene/EtOAc, 17:3): R_f = 0.5; mp: 118–120°C; $[\alpha]^{20}_D$ + 12 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.90–7.05 (m, 42 H, H arom.), 5.47 (s, 1 H, C*H*Ph), 5.46 (s, 1 H, C*H*Ph), 4.91 [d, ^2J 12.3 Hz, 1 H, CH_2(NAP)], 4.80 (d, 1 H, CH_2(NAP)], 4.75 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.57 (s, 1 H, C*H*Ph), 4.49 (d, ^2J 12.6 Hz, 1 H, CH_2Ph) and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.1, 164.8, 164.5 (C = O), 137.4, 137.4, 137.2, 136.8, 135.4, 133.4, 133.2, 133,1, 132,9 (C-*q*. arom.), 129.9–125.4 (C arom.), 101.9 (*C*HPh), 101.2 (*C*HPh), 100.6 (*C*HPh), 73.87 [*C*H_2(NAP)], 70.22 (*C*H_2Ph) and Table VI; ESI-MS: ($[M+Na]^+$, $C_{53}H_{70}O_{19}Na$): *m/z* calculated 1333.4409, found 1333.4407.

Compound ***14***

For compound **14** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside), the deprotection reaction was performed according to procedure B starting from **13** (621 mg, 4.7 mmol) in solvent (12.5 ml) and using DDQ (323 mg, 1.42 mmol). After stirring for 7 h, work-up and chromatography (toluene/EtOAc, 9:1'17:3) gave **13** (889 mg, 78%): TLC (toluene/EtOAc, 17:3): R_f = 0.3; mp: 138–140°C; $[\alpha]^{20}_D$ + 12 (*c* 0.75, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 8.02–7.06 (m, 35 H, H arom.), 5.47 (s, 1 H, C*H*Ph), 5.42 (s, 1 H, C*H*Ph), 4.76 (d, ^2J 12.6 Hz, 1 H, CH_2Ph), 4.62 (s, 1 H, C*H*Ph), 4.32 (d, 1 H, CH_2Ph), 2.64 (d, *J* 3.7 Hz, 1 H, OH-3c) and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.9, 164.8, 164.6 (C = O), 137.3, 137.2, 137.0, 136.8, 133.6, 133.3, 133.2 (C-*q* arom.), 130.1–125.4 (C arom.), 102.0 (*C*HPh), 101.8 (*C*HPh), 100.6 (*C*HPh), 70.3 (*C*H_2Ph) and Table VI; ESI-MS: ($[M+Na]^+$, $C_{67}H_{62}O_{19}Na$): *m/z* calculated 1193.3783, found 1193.3771; ($[M+K]^+$, $C_{67}H_{62}O_{19}K$): *m/z* calculated 1209.3522, found 1209.3505; ($[M-H+2Na]^+$, $C_{67}H_{61}O_{19}Na_2$): *m/z* calculated 1215.3602, found 1215.3547.

Compound ***15***

According to procedure A, the required tetrasaccharide **15** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-NAP-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) was obtained starting from donor **8** (323 mg, 0.58 mmol) in CH_2Cl_2 (10 ml), containing molecular sieves (1.0 g), trisaccharide **14** (617 mg, 0.53 mmol), NIS (142 mg, 0.63 mmol), and TESOTf (10 µl, 0.05 mmol). After 1 h stirring and work-up, a chromatographic purification (toluene/EtOAc, 19:1.9:1) yielded **15** (770 mg, 88%): TLC (toluene/EtOAc, 4:1): R_f = 0.5; mp: 132–134°C; $[\alpha]^{20}_D$ + 28 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.78–7.04 (m, 52 H, H arom.), 5.53 (s, 1 H, C*H*Ph), 5.43 (s, 1 H, C*H*Ph), 4.89 [d, ^2J 12.5 Hz, 1 H, CH_2(NAP)], 4.82 (s, 1 H, C*H*Ph), 4.79 [d, 1 H, CH_2(NAP)], 4.76 (d, ^2J 11.4 Hz, 1 H, CH_2Ph), 4.74 (s, 1 H, C*H*Ph), 4.50 (d, 1 H, CH_2Ph) and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.1, 164.7, 164.6, 164.5 (C = O), 137.4, 137.3, 137.2, 137.1, 136.7, 135.4, 133.4, 133.3, 133.1, 133.0, 132.9 (C-*q* arom.), 129.8–125.3 (C arom.), 101.8 (*C*HPh), 101.2 (*C*HPh), 101.1 (*C*HPh), 100.8 (*C*HPh), 73.8 [*C*H_2(NAP)], 70.2 (*C*H_2Ph) and Table VI. Analytical calculated for $C_{98}H_{88}O_{25}$: C, 70.66, H, 5.32; found: C, 70.57, H, 5.23.

Compound ***16***

The reaction was performed according to procedure B starting from **15** (3.79 g, 2.28 mmol) in solvent (55 ml) and using DDQ (1.55 mg, 6.83 mmol). After for 7.5 h at room temperature, work-up and chromatographic purification (toluene/EtOAc, 17:3) afforded the required compound **16** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) (2.78 g, 80%); TLC (toluene/EtOAc, 4:1): R_f = 0.3; mp: 162–164°C; $[\alpha]^{20}_D$ – 9 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.91–7.04 (m, 45 H, H arom.), 5.51 (s, 1 H, C*H*Ph), 5.39 (s, 1 H, C*H*Ph), 4.83 (s, 1 H, C*H*Ph), 4.75 [d, ^2J 10.8 Hz, 1 H, CH_2(NAP)], 4.74 (s, 1 H, C*H*Ph), 4.50 (d, 1 H, CH_2(NAP)], 2.73 (d, *J* 3.8 Hz, 1 H, OH-3d) and Table V; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.8, 164.7, 164.6, 164.6 (C = O), 137.3, 137.2, 137.1, 137.0, 136.7, 133.6, 133.4, 133.1, 133.0 (C-*q* arom.), 129.9–125.3 (C arom.), 101.8 (*C*HPh), 101.7 (*C*HPh), 101.1 (*C*HPh), 100.7 (*C*HPh), 70.2 (*C*H_2Ph) and Table VI. Analytical calculated for $C_{87}H_{80}O_{25}$: C, 68.50, H, 5.29; found: C, 68.09, H, 5.27.

Compound ***17***

The target pentasaccharide **17** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl-

(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) was synthesized as described in procedure A, starting from **8** (337 mg, 0.61 mmol) in CH_2Cl_2 (18 ml), containing molecular sieves (1.8 g), tetrasaccharide **16** (838 mg, 0.55 mmol), NIS (148 mg, 0.66 mmol) and $Sn(OTf)_2$ (23 µg, 0.06 mmol). After 2 h stirring, work-up and chromatography (toluene/EtOAc, 19:1, 9:1 and 17:3) enabled collecting **17** (820 mg, 74%); TLC (toluene/EtOAc, 4:1): R_f 0.6. mp: 162–165°C; $[\alpha]^{20}_D$ + 26 (*c* 1.0, CH_2Cl_2); ^{1}H=NMR ($CDCl_3$, 400 MHz): δ 7.85–7.04 (m, 62 H, H arom.), 5.51 (s, 1 H, C*H*Ph), 5.45 (s, 1 H, C*H*Ph), 5.36 (t, $J_{1e,2e}$ and $J_{2e,3e}$ 7.8 Hz, 1 H, H-2e), 5.14 (t, $J_{1c,2c}$ and $J_{2c,3c}$ 4.7 Hz, 1 H, H-2c*), 5.04 (d, 1 H, H-1e), 4.96 (t, $J_{1a,2a}$ and $J_{2a,3a}$ 8.4 H, 1 H, H-2a), 4.94 (d, 1 H, H-1c*), 4.92 (s, 1 H, C*H*Ph), 4.91 [d, ^2J 13.0 Hz, 1 H, CH_2(NAP)], 4.89 (d, $J_{1d,2d}$ 7.3 Hz, 1 H, H-1d*), 4.83 (t, $J_{1b,2b}$ and $J_{2b,3b}$ 4.3 Hz, 1 H, H-2b*), 4.81 (dd, $J_{2d,3d}$ 5.9 H, 1 H, H-2d*), 4.80 (d, 1 H, CH_2(NAP)], 4.79 (d, 1 H, H-1b*), 4.75 (d, ^2J 12.6 Hz, 1 H, CH_2Ph), 4.72 (s, 1 H, C*H*Ph), 4.69 (s, 1 H, C*H*Ph), 4.49 (d, 1 H, CH_2Ph), 4.46 (d, 1 H, H-1a), 4.33 (dd, $J_{5a,6a}$ 4.6 Hz, $J_{6a,6'a}$ 10.2 Hz, 1 H, H-6a), 4.21 (dd, $J_{5,6}$ 4.8 Hz, $J_{6,6'}$ 10.4 Hz, 1 H, H-6), 4.14–4.12 (m, 2 H, H-6), 4.09–3.99 (m, 2 H, H-3d*, H-6), 4.06 (t, $J_{3a,4a}$ 9.0 Hz, 1 H, H-3a), 4.01 (dd, $J_{3c,4c}$ 10.4 Hz, 1 H, H-3c*), 3.99 (t, $J_{4c,5c}$ 8.5 Hz, 1 H, H-4c*), 3.91–3.86 (m, 1 H, H-3b*), 3.89 (t, $J_{3e,4e}$ and $J_{4e,5e}$ 8.3 Hz, 1 H, H-4e), 3.86 (dd, $J_{3e,4e}$ 8.5 Hz, 1 H, H-3e), 3.72 (t, $J_{5,6'}$ and $J_{6,6'}$ 10.8 Hz, 1 H, H-6'), 3.69 (t, $J_{5a,6'a}$ and $J_{6a,6'a}$ 10.7 Hz, 1 H, H-6'a), 3.67 (t, $J_{3b,4b}$ and $J_{4b,5b}$ 8.4 Hz, 1 H, H-4b*), 3.59–3.36 (m, 8 H, H-5a, H-5b, H-5c, H-5d, H-5e, 3 H-6), 3.24 (t, $J_{3d,4d}$ and $J_{4d,5d}$ 8.6 Hz, 1 H, H-4d*), 3.20 (t, 1 H, H-4a, $J_{3a,4a}$ and $J_{4a,5a}$ 9.3 Hz), asterisks indicate that assignment of these signals to units b, c, and d can be inverted; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.1, 164.8, 164.7, 164.6, 164.5 (C = O), 137.4, 137.3, 137.2, 136.7, 135.4, 133.6, 133.5, 133.4, 133.1, 133.0, 132.8 (C-*q* arom.), 129.8–125.3 (C arom.), 102.0 (*C*HPh), 101.3 (*C*HPh), 101.1 (*C*HPh), 100.9 (*C*HPh), 100.6 (*C*HPh), 99.4 (C-1a), 98.6 (C-1e), 98.1 (C-1b*), 97.2 (C-1c*), 96.8 (C-1d*), 81.3 (C-4e), 78.7 (C-4a), 78.3 (C-3e), 78.0 (C-4c*), 77.9 (C-4d*), 77.3 (C-4b*), 76.6 (C-3c*), 74.5 (C-3a, C-3b*), 74.1 (C-2a, C-3d*), 73.8 (C-8e), 73.5 (C-2d*), 73.3 (C-2e), 72.9 (C-2b*), 72.4 (C-2c*), 70.2 (CH_2Ph), 68.7, 68.6, 68,5 (C-6a, C-6b, C-6c, C-6d, C-6e), 66.40 66.1, 65.6, 65.5, 65.4 (C-5a, C-5b, C-5c, C-5d, C-5e), asterisk indicates that assignment of these signals can be inverted. Analytical calculated for $C_{118}H_{106}O_{31}$: C, 70.16, H, 5.29; found: C, 70.23, H, 5.30.

*Compound **18***

The reaction was performed according to procedure B starting from **17** (2.0 g, 0.99 mmol) in solvent (20 ml) and using DDQ (675 mg, 2.97 mmol). After 5 h stirring and work-up, chromatographic purification (toluene/EtOAc, 17:3, 4:1) provided **18** (benzyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranoside) (1.60 g, 86%); TLC (toluene/EtOAc, 4:1): R_f = 0.4; mp: 176–179°C; $[\alpha]^{20}_D$ + 3 (*c* 1.0, CH_2Cl_2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.96–7.04 (m, 55 H, H arom.), 5.51 (s, 1 H, C*H*Ph), 5.42 (s, 1 H, C*H*Ph), 5.20 (dd, $J_{1e,2e}$ 7.6 Hz, $J_{2e,3e}$ 10.4 Hz, 1 H, H-2e), 5.18 (t, $J_{1c,2c}$ and $J_{2c,3c}$ 5.2 Hz, 1 H, H-2c*), 5.09 (d, 1 H, H-1e), 4.97 (d, 1 H, H-1c*), 4.94 (dd, $J_{1a,2a}$ 7.8 Hz, $J_{2a,3a}$ 8.4 Hz, 1 H, H-2a), 4.92–4.91 (m, 2 H, H-1d*, H-2d*), 4.90 (s, 1 H, C*H*Ph), 4.84 (t, $J_{1b,2b}$ and $J_{2b,3b}$ 4.5 Hz, 1 H, H-2b*), 4.80 (d, 1 H, H-1b*), 4.76 (d, ^2J 13.1 Hz, 1 H, CH_2Ph), 4.73 (s, 1 H, C*H*Ph), 4.68 (s, 1 H, C*H*Ph), 4.50 (d, 1 H, CH_2Ph), 4.46 (d, 1 H, H-1a), 4.33 (dd, $J_{5,6}$ 4.7 Hz, $J_{6,6'}$ 10.3 Hz, 1 H, H-6), 4.21 (dd, $J_{5,6}$ 4.9 Hz, $J_{6,6'}$ 10.4 Hz, 1 H, H-6), 4.13–3.88 (m, 9 H, H-3a, H-3b, H-3c, H-3d, H-3e, H-4c*, 3 H-6), 3.67 (t, $J_{3c,4c}$ and $J_{4c,5c}$ 8.7 Hz, 1 H, H-4c*), 3.65 (t, $J_{3e,4e}$ and $J_{4e,5e}$ 9.3 Hz, 1 H, H-4e), 3.72–3.34 (m, 10 H, H-5a, H-5b, H-5c, H-5d, H-5e, 5 H-6), 3.23 (t, $J_{3d,4d}$ and $J_{4d,5d}$ 10.0 Hz, 1 H, H-4d*), 3.20 (t, $J_{3a,4a}$ and $J_{4a,5a}$ 9.5 Hz, 1 H, H-4a), 2.69 (d, *J* 3.4 Hz, 1 H, OH), asterisks indicate that assignment of these signals to units b, c, and d can be inverted; ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 165.9, 164.8 164.7, 164.6, 164.5 (C = O), 137.3, 137.2, 137.0, 136.7, 133.8, 133.6, 133.5, 133.2 (C-*q* arom.), 129.9–125.3 (C arom.), 102.0 (*C*HPh), 101.8 (*C*HPh), 101.4 (*C*HPh), 100.9 (*C*HPh), 100.6 (*C*HPh), 99.4 (C-1a), 98.4 (C-1e), 98.1 (C-1b*), 97.2 (C-1c*), 96.8 (C-1d*), 80.8 (C-4e), 78.7 (C-4a), 78.0 (C-4c*, C-4d*), 77.3 (C-4b*), 76.6 (C-3c*), 74.7 (C-2e), 74.5 (C-3a, C-3b*), 74.3 (C-3d*), 74.1 (C-2a), 73.4 (C-2d*), 72.9 (C-2b*), 72.5 (C-3e), 72.4 (C-2c*), 70.2 (CH_2Ph), 68.7, 68.6, 68.5 (C-6a, C-6b, C-6c, C-6d, C-6e), 66.4, 66.0, 65.6, 65.4 (C-5a, C-5b, C-5c, C-5d, C-5e). Analytical calculated for $C_{107}H_{98}O_{31}$: C, 68.36, H, 5.25; found: C, 68.05; H, 5.25.

Deprotection steps

Removal of benzylidene groups. To a solution of oligosaccharide (1 equiv.) in a acetone/MeOH/water (1:4:1, v/v/v) mixture was added an acid (1 equiv.) and the media was heated to 70°C. When TLC monitoring indicated completion of the reaction, the mixture was cooled to room temperature and neutralized with triethyamine; the solvents were coevaporated with ethanol. The residue was then dissolved in MeOH and poured onto cold light petroleum or cold toluene. The resulting solid was filtered off, washed, and dried. The expected compound was finally purified by flash chromatography.

Debenzoylation. Sodium (0.2 equiv.) was added to a solution of previously obtained oligosaccharide (1 equiv.) in MeOH. The mixture was heated to 50°C, neutralized with acetic acid after completion of the reaction, and concentrated. The crude residue was further dissolved in water and methyl benzoate removed with CH_2Cl_2. The aqueous layer was then coevaporated with ethanol and the product purified by size exclusion chromatography (SEC) over Sephadex G-15 gel eluting with water. The collected fractions were finally freeze-dried to afford the target benzyl oligoglucoside.

Debenzylation. In a MeOH/water mixture (1:1, v/v) were successively introduced the oligosaccharide (1 equiv.) and palladium acetate. After vigorously stirring for 2 h at room temperature under a hydrogen atmosphere, palladium acetate (10% w) was added again and stirring under hydrogen was kept on 2 h more. After filtration on sintered glass, the

organic layer was coevaporated with absolute ethanol. The final product was purified by gel permeation over Sephadex G-15 (water), and freeze-drying of the collected fractions provided the pure free oligosaccharide.

Compound ***19***

According to procedure C, compound **14** (2.16 g, 1.85 mmol) in solvent (84 mL) was treated with camphorsulfonic acid (CSA, 429 mg, 1.85 mmol) for 7 h. Work-up and chromatography (CH_2Cl_2/MeOH, 9/1) afforded **19** (benzyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-β-D-glucopyranoside) (1.29 g, 77%); TLC (CH_2Cl_2/MeOH, 17:3): R_f = 0.4; mp: 176–178°C; $[\alpha]^{20}_D$ + 15 (*c* 1.0, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.52–6.88 (m, 20 H, H arom.), 4.62 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.42 (d, 1 H, CH_2Ph) and Table V; ^{13}C-NMR (CD_3OD, 100 MHz): δ 167.2, 166.2, 166.0 (C = O), 138.5, 134.3, 134.1, 133.9 (C-*q* arom.), 130.9–128.6 (C arom.), 71.4 (*C*H_2Ph) and Table VI; ESI-MS: ($[M+Na]^+$, $C_{46}H_{50}O_{19}Na$): *m/z* calculated 929.2844, found 929.2841; ($[M+K]^+$, $C_{46}H_{50}O_{19}K$): *m/z* calculated 945.2583, found 945.2523.

Compound ***20***

According to procedure C, compound **16** (2.88 g, 1.89 mmol) in solvent (84 ml) was submitted to deacetalyzation (CSA: 439 mg, 1.89 mmol) for 3 h. Work-up and chromatography (CH_2Cl_2/MeOH, 9:1) afforded **20** (benzyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-4β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-β-D-glucopyranoside) (1.62 g, 73%); TLC (CH_2Cl_2/MeOH, 17:3): R_f = 0.4; mp: 152–154°C; $[\alpha]^{20}_D$ + 20 (*c* 1.0, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.50–6.88 (m, 25 H, H arom.), 4.79 (d, $J_{1a,2a}$ and $J_{2a,3a}$ 8.7 Hz, 1 H, H-2a), 4.74 (t, $J_{1,2}$ and $J_{2,3}$ 8.8 Hz, 1 H, H-2b or H-2c), 4.72 (t, $J_{1,2}$ and $J_{2,3}$ 8.7 Hz, H-2b or H-2c), 4.71 (t, $J_{1d,2d}$ and $J_{2d,3d}$ 8.6 Hz, 1 H, H-2d), 4.62 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.57 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b or H-1c), 4.47 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b or H-1c), 4.41 (d, 1 H, CH_2Ph), 4.40 (d, 1 H, H-1a), 4.37 (d, 1 H, H-1d), 3.82–3.69 (m, 6 H, H-3a, H-3b or H-3c, H-6), 3.62–3.52 (m, 5 H, H-3b or H-3c, H-6), 3.38–3.05 (m, 9 H, H-3d, H-4a, H-4b, H-4c, H-4d, H-5a, H-5b, H-5c, H-5d); ^{13}C-NMR (CD_3OD, 100 MHz): δ 167.1, 166.1, 166.0, 165.8 (C = O), 138.4, 134.3, 134.2, 134.1, 133.9 (C-*q* arom.), 130.8–128.6 (C arom.), 102.2 (C-1b or C-1c), 101.8, 101.7 (C-1d and C-1b or C-1c), 100.9 (C-1a), 83.9 (C-3a), 82.8, 82.4 (C-3b, C-3c), 78.3, 78.0, 77.8 (C-5a, C-5b, C-5c, C-5d), 76.1 (C-3d), 75.2 (C-2d), 74.6, 74.5, 74.3 (C-2a, C-2b, C-2c), 71.5 (C-4d), 71.4 (*C*H_2Ph), 70.0, 69.8, 69.7 (C-4a, C-4b, C-4c), 62.5, 62.4, 62.4 (C-6a, C-6b, C-6c, C-6d); ESI-MS: ($[M+Na]^+$, $C_{59}H_{64}O_{25}Na$): *m/z* calculated 1195.3634, found 1195.3627; ($[M+K]^+$, $C_{59}H_{64}O_{25}K$): *m/z* calculated 1211.3374, found 1211.3461.

Compound ***21***

According to procedure C, compound **18** (2.10 g, 1.12 mmol) in solvent (60 ml) was treated with PTSA, H_2O (213 mg, 1.12 mmol) during 4 h. Work-up followed by chromatographic purification (CH_2Cl_2/MeOH, 9:1) afforded **21** (benzyl 2-*O*-benzoyl-β-D-glucopyranosyl-(1,3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1.3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1.3)-2-*O*-benzoyl-4β-D-glucopyranosyl-(1.3)-2-*O*-benzoyl-β-D-glucopyranoside) (1.14 g, 76 %): TLC (CH_2Cl_2/MeOH, 17:3): R_f = 0.3; mp: 171–174°C; $[\alpha]^{20}_D$ + 21 (*c* 1.0, MeOH); ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.48–6.87 (m, 30 H, H arom.), 4.79–4.52 (m, 5 H, H-2a, H-2b, H-2c, H-2d, H-2e), 4.60 (d, ^2J 12.5 Hz, 1 H, CH_2Ph), 4.53 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.39 (d, $J_{1,2}$ 7.8 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.39 (d, 1 H, CH_2Ph), 4.38 (d, $J_{1a,2a}$ 7.8 Hz, 1 H, H-1a), 4.31 (d, $J_{1,2}$ 8.2 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.29 (d, $J_{1,2}$ 8.3 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 3.79–3.72 (m, 6 H, H-3, H-6), 3.67–3.43 (m, 8 H, H-3, H-6), 3.36–3.02 (m, 9 H, H-3e, H-4a, H-4b, H-4c, H-4d, H-4e, H-5a, H-5b, H-5c, H-5d, H-5e); ^{13}C-NMR (CD_3OD, 100 MHz): δ 167.1, 166.1, 165.9, 165.8, 165.8 (C = O), 138.4, 134.4, 134.3, 134.2, 134.1, 133.9 (C-*q* arom.), 130.8–128.5 (C arom.), 102.2, 101.9, 101.8, 101.7 (C-1b, C-1c, C-1d, C-1e), 100.9 (C-1a), 83.9 (C-3a), 82.8, 82.4, 82.3 (C-3b, C-3c, C-3d), 78.4, 78.1, 77.9, 77.8 (C-5a, C-5b, C-5c, C-5d, C-5e), 76.1 (C-3e), 75.2 (C2-e), 74.6, 74.4, 74.3 (C-2a, C-2b, C-2c, C-2d), 71.5 (C-4e), 71.4 (*C*H_2Ph), 70.0, 69.8, 69.7, 69.6 (C-4a, C-4b, C-4c, C-4d), 62.6, 62.5, 62.4 (C-6a, C-6b, C-6c, C-6d, C-6e); ESI-MS: ($[M+Na]^+$, $C_{72}H_{78}O_{31}Na$): *m/z* cal-culated 1461.4425, found 1461.4413; ($[M+K]^+$, $C_{72}H_{78}O_{31}K$): *m/z* calculated 1477.4164, found 1477.4237; ($[M-H+2Na]^+$, $C_{72}H_{77}O_{31}Na_2$): *m/z* calculated 1483.4244, found 1483.4241.

Compound ***22***

This compound was obtained as described in procedure D, starting from **19** (1.97 g, 2.17 mmol) in MeOH (60 ml) and sodium (75 mg, 3.26 mmol). After 8 h stirring, work-up and chromatography, **22** (benzyl β-D-glucopyranosyl-(1,3)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranoside) (1.29 mg) was isolated in quantitative yield; TLC (EtOAc/*i*-PrOH/H_2O, 3:3:1): R_f = 0.6; mp: 176–178°C; $[\alpha]^{20}_D$ – 29 (*c* 1.0, water); ^{1}H-NMR (D_2O, 400 MHz): δ 7.34–7.26 (m, 5 H, H arom.), 4.81 (d, ^2J 11.6 Hz, 1 H, CH_2Ph), 4.63 (d, $J_{1,2}$ 7.9 Hz, 1 H, H-1b or H-1c), 4.62 (d, 1 H, CH_2Ph), 4.61 (d, $J_{1,2}$ 7.9 Hz, 1 H, H-1b or H-1c), 4.42 (d, $J_{1a,2a}$ 8.0 Hz, 1, H-1a), 3.81–3.76 (m, 3 H, H-6), 3.65–3.55 (m, 5 H, H-3a, H-3b, 3 H-6), 3.42–3.19 (m, 10 H, H-2a, H-2b, H-2c, H-3c, H-4a, H-4b, H-4c, H-5a, H-5b, H-5c); ^{13}C-NMR (D_2O, 100 MHz): δ 136.7 (C-*q* arom.), 129.1, 129.0, 128.8 (C arom.), 103.1, 102.8 (C-1b, C-1c), 101.2 (C-1a), 84.5, 84.3 (C-3a, C-3b), 76.3, 75.9, 75.8 (C-3c, C-5a, C-5b, C-5c), 73.7, 73.5, 73.2 (C-2a, C-2b, C-2c), 71.8 (*C*H_2Ph), 69.8 (C-4c), 68.4, 68.3 (C-4a, C-4b), 60.9 (C-6a, C-6b, C-6c); ESI-MS: ($[M+Na]^+$, $C_{25}H_{38}O_{16}Na$): *m/z* calculated 617.2058, found 617.2060; ($[M+K]^+$, $C_{25}H_{38}O_{16}K$): *m/z* calculated 633.1797, found 633.1801; ($[M-H+2Na]^+$, $C_{25}H_{37}O_{16}Na_2$): *m/z* calculated 639.1877, found 639.1876.

Compound ***23***

This compound was prepared as disclosed in procedure D starting from **20** (2.29 g, 1.96 mmol) in MeOH (70 ml) and using sodium (67 mg, 2.91 mmol). After 8 h stirring, work-up and chromatography afforded **23** (benzyl β-D-glucopyranosyl-(1,3)-4β-D-glucopyranosyl-(1,3)-β-D-glucopyranosyl-(1.3)-?β-D-glucopyranoside) (1.47 g) in 99% yield; TLC

(EtOAc/*i*-PrOH/H_2O, 3:3:1): R_f = 0.5; $[\alpha]^{20}_D$ – 29 (*c* 1.0, water); ^{1}H-NMR (D_2O, 400 MHz): δ 7.35–7.26 (m, 5 H, H arom.), 4.81 (d, ^2J 11.6 Hz, 1 H, CH_2Ph), 4.65 (d, $J_{1,2}$ 8.2 Hz, 1 H, H-1b, H-1c or H-1d), 4.62 (d, $J_{1,2}$ 8.6 Hz, 1 H, H-1b, H-1c or H-1d), 4.62 (d, 1 H, CH_2Ph), 4.61 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b, H-1c or H-1d), 4.42 (d, $J_{1a,2a}$ 8.0 Hz, 1 H, H-1a), 3.81–3.76 (m, 4 H, H-6), 3.66–3.56 (m, 7 H, H-3a, H-3b, H-3c, H-6), 3.43–3.19 (m, 13 H, H-2a, H-2b, H-2c, H-2d, H-3d, H-4a, H-4b, H-4c, H-4d, H-5a, H-5b, H-5c, H-5d); ^{13}C-NMR (D_2O, 100 MHz): δ 136.7 (C-*q* arom.), 129.1, 129.0, 128.8 (C arom.), 103.1, 102.8, 102.7 (C-1b, C-1c, C-1d), 101.2 (C-1a), 84.5, 84.40 84.2 (C-3a, C-3b, C-3c), 76.3, 75.9, 75.8 (C-3d, C-5a, C-5b, C-5c, C-5d), 73.7, 73.6, 73.5, 73.2 (C-2a, C-2b, C-2c, C-2d), 71.8 (*C*H_2Ph), 69.8 (C-4d), 68.5, 68.4, 68.3 (C-4a, C-4b, C-4c), 60.9 (C-6a, C-6b, C-6c, C-6d); ESI-MS: ($[M+Na]^+$, $C_{31}H_{48}O_{21}Na$): *m/z* calculated 779.2586, found 779.2580; ($[M+K]^+$, $C_{31}H_{48}O_{21}K$): *m/z* calculated 795.2325, found 795.2380.

*Compound **24***

Debenzoylation was performed according to procedure D and starting from **21** (1.62 g, 1.13 mmol) in MeOH (54 ml) with sodium (39 mg, 1.70 mmol). After 7 h stirring and work-up, SEC purification provided target **24** (benzyl β-D-glucopyranosyl-(1,3)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranosyl-(1,3)-β-D-glucopyranoside) (1.03 mg, 99%); TLC (EtOAc/*i*-PrOH/H_2O, 3:3:1): R_f = 0.4; $[\alpha]^{20}_D$ – 21 (*c* 1.0, water); ^{1}H-NMR (D_2O, 400 MHz): δ 7.35–7.28 (m, 5 H, H arom.), 4.81 (d, ^2J 11.6 Hz, 1 H, CH_2Ph), 4.66 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.65 (d, $J_{1,2}$ 8.4 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.63 (d, $J_{1,2}$ 9.0 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.63 (d, 1 H, CH_2Ph), 4.61 (d, $J_{1,2}$ 7.4 Hz, 1 H, H-1b, H-1c, H-1d or H-1e), 4.41 (d, $J_{1a,2a}$ 8.0 Hz, 1 H, H-1a), 3.81–3.76 (m, 5 H, H-6), 3.66–3.56 (m, 9 H, H-3a, H-3b, H-3c, H-3d, H-6), 3.43–3.20 (m, 16 H, H-2a, H-2b, H-2c, H-2d, H-2e, H-3e, H-4a, H-4b, H-4c, H-4d, H-4e, H5-a, H-5b, H-5c, H-5d, H-5e); ^{13}C-NMR (D_2O, 100 MHz): δ 136.7 (C-*q* arom.), 129.1, 129.0, 128.8 (C arom.), 103.1, 102.8 (C-1b, C-1c, C-1d, C-1e), 101.2 (C-1a), 84.5, 84.4, 84.2 (C-3a, C-3b, C-3c, C-3d), 76.3, 75.9, 75.8 (C-3e, C-5a, C-5b, C-5c, C-5d, C-5e), 73.7, 73.6, 73.5, 73.2 (C-2a, C-2b, C-2c, C-2d, C-2e), 71.8 (C-7), 69.8 (C-4e), 68.5, 68.4, 68.3 (C-4a, C-4b, C-4c, C-4d), 60.9 (C-6a, C-6b, C-6c, C-6d, C-6e); ESI-MS: ($[M+Na]^+$, $C_{37}H_{58}O_{26}Na$): *m/z* calculated 941.3114, found 941.3114; ($[M+K]^+$, $C_{37}H_{58}O_{26}K$): *m/z* calculated 957.2853, found 957.2821; : ($[M-H+2Na]^+$, $C_{37}H_{57}O_{26}Na_2$): *m/z* calculated 963.2934, found 963.2939.

*Laminaritriose **(2)***

The reaction was performed according to procedure E starting from **22** (850 mg, 1.04 mmol) in solvent (20 ml) and in the presence of palladium acetate (85 mg, 0.38 mmol, twice). After stirring for 3.5 h, work-up, and SEC, **2** (0.50 g) was isolated in 95% yield; TLC (EtOAc/*i*-PrOH/H_2O, 3:3:2): R_f = 0.3; ^{1}H-NMR (D_2O, 400 MHz): δ 5.12 (d, $J_{1a\alpha,2a\alpha}$ 3.7 Hz, 0.45 H, H-1aα), 4.66 (d, $J_{1,2}$ 8.6 Hz, 1 H, H-1), 4.65 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1), 4.56 (d, $J_{1a\beta,2a\beta}$ 8.0 Hz, 0.55 H, H-1aβ), 3.83–3.59 (m, 9 H), 3.47–3.23 (m, 9 H); ^{13}C-NMR (D_2O, 100 MHz): δ 103.1, 102.9, 102.8 (C-1b, C-1c), 96.0 (C-1aβ), 92.3 (C-1aα), 84.7, 84.5, 84.4, 82.5 (C-3a, C-3b), 76.3, 75.9, 75.8, 74.2, 73.7, 73.6, 73.5, 71.5, 71.4, 69.9, 68.4, 68.3, 61.0, 60.8 (C-6). ESI-MS: ($[M+Na]^+$, $C_{18}H_{32}O_{16}Na$): *m/z* calculated 527.1588, found 527.1588; ($[M+K]^+$, $C_{18}H_{32}O_{16}K$): *m/z* calculated 543.1327, found 543.1367.

*Laminaritetraose **(3)***

The required tetrasaccharide was synthesized as disclosed in procedure E with **23** (1.12 g, 1.48 mmol), solvent (20 ml) and catalyst (112 mg, 0.05 mmol, twice). After stirring for 5 h, work-up and chromatographic purification gave **3** (0.95 g, 96%): TLC (EtOAc/*i*-PrOH/H_2O, 3:3:2): R_f = 0.2; ^{1}H-NMR (D_2O, 400 MHz): δ 5.13 (d, $J_{1a\alpha,2a\alpha}$ 3.7 Hz, 0.36 H, H-1aα), 4.67 (d, $J_{1,2}$ 8.9 Hz, 2 H, H-1), 4.65 (d, $J_{1,2}$ 7.8 Hz, 1 H, H-1), 4.57 (d, $J_{1a\beta,2a\beta}$ 8.0 Hz, 0.64 H, H-1aβ), 3.83–3.59 (m, 12 H), 3.47–3.23 (m, 12 H); ^{13}C-NMR (D_2O, 100 MHz): δ 103.1, 103.0, 102,9, 102,8 (C-1b, C-1c, C-1d), 96.0 (C-1aβ), 92.3 (C-1aα), 84.7, 84.5, 84.3, 84.2, 82.5 (C-3a, C-3b, C-3c), 76.3, 75.9, 75.8, 74.1, 73.7, 73.6, 73.5, 71.5, 71.4, 69.9, 68.4, 68.3, 61.0, 60.8 (C-6); ESI-MS: ($[M+Na]^+$, $C_{24}H_{42}O_{21}Na$): *m/z* calculated 689.2116, found 689.2121; ($[M+K]^+$, $C_{24}H_{42}O_{21}K$): *m/z* calculated 705.1856, found 705.1843.

*Laminaripentaose **(4)***

Final deprotection was performed according to procedure E starting from **24** (960 mg, 1.04 mmol) in solvent (20 ml) and using palladium acetate (192 mg, 0.43 mmol, twice). After 2.5 h stirring, work-up and SEC purification gave the required pentasaccharide **26** (0.81 g) in 94% yield. TLC (EtOAc/*i*-PrOH/H_2O, 3:3:2): R_f = 0.2; ^{1}H-NMR (D_2O, 400 MHz): δ 5.12 (d, $J_{1a\alpha,2a\alpha}$ 3.8 Hz, 0.46 H, H-1aα), 4.68 (d, $J_{1,2}$ 8.0 Hz, 1 H, H-1), 4.68 (d, $J_{1,2}$ 8.1 Hz, 1 H, H-1), 4.66 (d, $J_{1,2}$ 7.8 Hz, 1 H, H-1), 4.64 (d, $J_{1,2}$ 7.7 Hz, 1 H, H-1), 4.56 (d, $J_{1a\beta,2a\beta}$ 8.0 Hz, 0.54 H, H-1aβ), 3.82–3.58 (m, 15 H), 3.47–3.23 (m, 15 H); ESI-MS: ($[M+Na]^+$, $C_{30}H_{52}O_{26}Na$): *m/z* calculated 851.2645, found 851.2650; ($[M-H+2Na]^+$, $C_{30}H_{51}O_{26}Na_2$): *m/z* calculated 873.2464, found 873.2525.

Animals

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

β-(1,3)-glucan

Phycarine was extracted and purified from the marine brown alga *Laminaria digitata* as described in Vetricka and Yvin (2004).

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 heparine (5 IU/ml; Sigma, St.Louis, MO). Phagocytosis of HEMA particles has been done as previously described (Vetricka and Yvin 2004). 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 engulfted particles were considered positive.

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 (Vetricka and Yvin, 2004).

IL-β assay

BALB/c mice were intraperitoneally injected with 100 μg oligosaccharides. Control mice obtained phosphate buffered saline (PBS) only. After various time intervals (30, 60, and 90 min, respectively), the mice were sacrificed, and blood was collected in Eppendorf tubes. Subsequently, the serum was prepared, collected and stored at –80°C for no more than 1 week.

The levels of IL-1β in serum samples were evaluated using a commercial kit OptEIA Mouse IL-1β Set (Pharmingen, San Diego, CA) according to the manufacturer's instructions. The optical density was determined using a STL enzyme-linked immunosorbent assay reader (Tecan U.S., Research Triangle Park, NC) at 450 nm with a correction at 570 nm. Data shown in Figure 6 were calculated from the standard curve prepared by the automated data reduction using linear regression analysis. A standard curve was run with each assay.

Cell lines

The BALB/c mouse-derived mammary tumor cell line Ptas64 was generously provided by Dr. Wei-Zen Wei of the Michigan Cancer Foundation (Wayne State University, Detroit, MI).

Tumor inhibition in vivo

Mice were injected directly into the mammary fat pads with 1×10^6/mouse of Ptas64 cells in PBS. The experimental treatment was begun after palpable tumors were found (usually 14 days after injection of cells) and after mice were assigned to experimental groups. Experimental treatment was achieved by daily IP injections of either phycarine or oligosaccharides diluted in PBS. After 2 weeks of treatment, the mice were sacrificed, and tumors were removed and weighed.

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Abbreviations

COSY, correlation spectroscopy; CSA, camphorsulfonic acid; DDQ, 2,3-dichloro-5,6-dicyano-1,4-quinone; ESI-MS, electrospray ionization mass spectrometry; HEMA, 2-hydroxyethyl methacrylate; NAP, 2-naphthylmethyl; NIS, *N*-iodosuccinimide; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; PBS, phosphate buffered saline; SEC, size exclusion chromatography; TESTOf, triethylsilyl trifluoromethanesulfonate; TLC, thin-layer chromatography.

References

Abel, G., Szolosi, J., Chihara, G., and Fachet, J. (1989) Effect of lentinan and mannan on phagocytosis of fluorescent latex microbeads by mouse peritoneal macrophages: a flow cytometric study. *Int J. Immunopharmacol.*, **11**, 615–621.

Amaya, T., Tanaka, H., Yamaguchi, T., Shibuya, N., and Takahashi, T. (2001) The first synthesis of tetraglucosyl glucitol having phytoalexin-elicitor activity in rice cells based on a sequential glycosylation strategy. *Tetrahedron Lett.*, **42**, 9191–9194.

Bentley, R. (1972) Configurational and conformational aspects of carbohydrate biochemistry. *Ann. Rev. Biochem.*, **41**, 953–996.

Black, W.A.P., Cornhill, W.J., Dewar, E.T., and Woodward, F.N. (1951) Manufacture of algal chemicals. III. Laboratory scale isolation of laminarin from brown marine algal. Vol. 1. *J. Appl. Chem.*, 505–517.

Bohn, J.A. and BeMiller, J.N. (1995) β-D-(1,3)-Glucans as biological response modifiers: a review of structure-functional activity relationships. *Carbohydr. Polym.*, **28**, 3–14.

Borbás, A., Szabó, S., Szilágyi, L., Bényei, A., and Lipták, A. (2002) Stereoselective (2-naphthyl)methylation of sugar hydroxyls by the hydrogenolysis of diastereoisomeric dioxolane-type (2-naphthyl)methylene acetals. *Carbohydr. Res.*, **337**, 1941–1951.

Brown, G.D. and Gordon, S. (2001) Immune recognition: a new receptor for β-glucans. *Nature*, **413**, 36–37.

de Nooy, A.E.J., Rori, V., Mmasci, G., Dentini, M., and Crescenzi, V. (2000) Synthesis and preliminary characterisation of charged derivatives and hydrogels from scleroglucan. *Carbohydr. Res.*, **324**, 116–126.

Dong, Q., Yao, J., Yang, X.T., and Fang, J.N. (2002) Structural characterization of a water-soluble β-D-glucan from fruiting bodies of *Agaricus blazei* Murr. *Carbohydr. Res.*, **337**, 1417–1421.

Falch, B.H., Espevik, T., Ryan, L., and Stokke, B.T. (2000) The cytokine stimulating activity of (1,3)-β-D-glucans is dependent on the triple helix conformation. *Carbohydrate Res.*, **329**, 587–596.

Gaunt, M.J., Yu, J., and Spencer, J.B. (1998) Rational design of benzyl-type protecting groups allows sequential deprotection of hydroxyl groups by catalytic hydrogenolysis. *J. Org. Chem.*, **63**, 4172–4173.

Gelin, M., Ferrières, V., and Plusquellec, D. (2000) A general and diastereoselective synthesis of 1,2-*cis*-hexofuranosides from **1**,2-*trans*-thiofuranosyl donors. *Eur. J. Org. Chem.*, 1423–1431.

He, H., Yang, F., and Du, Y. (2002) Synthesis of natural β-D-(1,3)-glucopyranosyl oligosaccharides. *Carbohydr. Res.*, **337**, 1673–1678.

Hofer, M. and Popísil, M. (1997) Glucan as stimulator of hematopoiesis in normal and gamma-irradiated mice. A survey of the authors' results. *Int. J. Immunopharmacol.*, **19**, 607–609.

Jamois, F. (2003) Oligo-β-(1,3)-glucans and derivatives: chemical synthesis, industrial procedures and biological properties. PhD diss., University of Rennes 1.

Kimura, Y., Tojima, H., Fukase, S., and Takeda, K. (1994) Clinical evaluation of sizofilan as assistant immunotherapy in treatment of head and neck cancer. *Acta Otolaryngol.*, **511**, 192–195.

Kobayashi, Y., Tanaka, H., and Ogasawara, N. (1974) Multiple 1,3-β-glucanases in the lytic enzyme complex of *Bacillus circulans* WL-12. *Agric. Biol. Chem.*, **38**, 973–978.

Kondo, Y., Kato, A., Hojo, H., Nozoe, S., Takeuchi, M., and Ochi, K. (1992) Cytokine-related immunopotentiating activities of paramylon, a β-(1,3)-D-glucan from *Euglena gracilis*. *J. Pharmacobio-Dyn.*, **5**, 617–621.

Lépagnol-Descamps, V., Richard, C., Lahaye, M., Potin, P., Yvin, J.C., and Kloareg, B. (1998) Purification and determination of the action pattern of *Haliotis tuberculata* laminarinase. *Carbohydr. Res.*, **310**, 283–289.

Lowman, D.W., Ferguson, D.A. Jr., and Williams, D.L. (2003) Structural characterization of (1,3)-β-D-glucans isolated from blastospore and hyphal forms of *Candida albicans*. *Carbohydr. Res.*, **338**, 1491–1496.

Miyanishi, N., Iwamoto, Y., Watanabe, E., and Odaz, T. (2003) Induction of TNF-α production from human peripheral blood monocytes with

β-(1,3)-glucan oligomer prepared from laminarin with β-(1,3)-glucanase from *Bacillus clausii* NM-1. *J. Biosci. Bioeng.*, **95**, 192–195.

Ning, J., Yi, Y., and Kong, F. (2002) A general strategy for the synthesis of 3,6-branched gluco-oligosaccharides: facile synthesis of the phytoalexin elicitor oligosaccharides. *Tetrahedron Lett.*, **43**, 5545–5549.

Ning, J., Zhang, W., Yi, Y., Yang, G., Wu, Z., Yi, J., and Kong, F. (2003) Synthesis of β-(1,6)-branched β-(1,3) glucohexaose and its analogues containing an β-(1,3) linked bond with antitumor activity. *Bioorg. Med. Chem.*, **11**, 2193–2203.

Ohmura, Y., Matsunaga, K., Motokawa, I., Sakurai, A., and Ando, T. (2001) Protective effects of a protein-bound polysaccharide, PSK, on *Candida albicans* infection in mice via tumor necrosis factor-alpha induction. *Int. Immunopharmacol.*, **1**, 1797–1811.

Read, S.M., Currie, G., and Bacic, A. (1996) Analysis of the structural heterogeneity of laminarin by electrospray-ionisation-mass spectrometry. *Carbohydr. Res.*, **281**, 187–201.

Ross, G. D., Vetvicka, V., Yan, J., Xia, Y., and Vetvicková, J. (1999) Therapeutic intervention with complement and β-glucan in cancer. *Immunopharmacol.*, **42**, 61–74.

Sarkar, A.K., Rostand, K.S., Jain, R.K., Matta, K.L., and Esko, J.D. (1997) Fucosylation of disaccharide precursors of sialyl Lewis^X inhibit selectin-mediated cell adhesion. *J. Biol. Chem.*, **272**, 25608–25616.

Sarkar, A.K., Brown, J.R., and Esko, J.D. (2000) Synthesis and glycan priming activity of acetylated disaccharides. *Carbohydr. Res.*, **329**, 287–300.

Schmid, F., Stone, B.A., McDougall, B.M., Bacic, A., Martin, K.L., Brownlee, R.T.C., Chai, E., and Seviour, R.J. (2001) Structure of epiglucan, a highly side-chain/branched (1,3;1,6)-β-glucan from the micro fungus *Epicoccum nigrum* Ehrenb. ex Schlecht. *Carbohydr. Res.*, **331**, 163–171.

Seljelid, R., Figenschau, Y., Bogwald, J., Rasmussen, L.T., and Austgulen, R. (1989) Evidence that tumor necrosis induced by aminated β-(1,3)-D-polyglucose is mediated by a concerted action of local and systemic cytokines. *Scand. J. Immunol.*, **30**, 687–694.

Sharp, J.K., Albersheim, P., Ossovski, P., Pilotti, A., Garegg, P., and Lindberg, P. (1984a) Comparison of the structures and elicitor activities of a synthetic and a mycelial-wall-derived hexa-(β-D-glucopyranosyl)-D-glucitol. *J. Biol. Chem.*, **18**, 11341–11345.

Sharp, J.K., Valent, B., and Albersheim, P. (1984b) Purification and partial characterization of a β-glucan fragment that elicits phytoalexin accumulation in soybean. *J. Biol. Chem.*, **18**, 11312–11320.

Takahashi, T., Okano, A., Amaya, T., Tanaka, T., and Doi, T. (2002) Solid-phase synthesis of a phytoalexin elicitor-active tetraglucosyl glucitol. *Synlett*, 911–914.

Takeo, K. and Tei, S. (1986) Synthesis of the repeating units of *Schizophyllan*. *Carbohydr. Res.*, **145**, 293–306.

Takeo, K., Maki, M., Wada, Y., and Kitamura, S. (1993) Synthesis of the laminara-oligosaccharide methyl β-glycosides of dp 3–8. *Carbohydr. Res.*, **245**, 81–96.

Tanabe, H., Imai, N., and Takechi, K. (1990) Studies of usefulness of postoperative adjuvant chemotherapy with lentinan in patients with gastrointestinal cancer. *J. Jpn. Soc. Cancer Ther.*,**25**, 1657–1667.

Tanaka, H., Amaya, T., and Takahashi, T. (2003) Parallel synthesis of multi-branched oligosaccharides related to elicitor active pentasaccharide in rice cell based on orthogonal deprotection and glycosylation reaction. *Tetrahedron Lett.*, **44**, 3053–3057.

Vetvicka, V. and Fornusek, L. (1987) Polymer microbeads in immunology. *Biomaterials*, **8**, 341–345.

Vetvicka, V. and Yvin, J.C. (2004) Effects of marine β-(1,3)-glucan on immune reactions. *Int. Immunopharmacol.*, **4**, 721–730.

Viladot, J.L., Stone, B., Driguez, H., and Planas, A. (1998) Expeditious synthesis of a new hexasaccharide using transglycosylation reaction catalyzed by *Bacillus* (**1**,3),(1,4)-β-D-glucan 4-glucanohydrolase. *Carbohydr. Res.*, **311**, 95–99.

Wright, J.A., Yu, J., and Spencer, J.B. (2001) Sequential removal of the benzyl-type protecting groups PMB and NAP by oxidative cleavage using CAN and DDQ. *Tetrahedron Lett.*, **42**, 4033–4036.

Xia, J., Abbas, S.A., Locke, R.D., Piskorz, C.F., Alderfer, J.L., and Matta, K.L. (2000) Use of 1,2-dichloro 4,5-dicyanoquinone (DDQ) for cleavage of the 2-naphthylmethyl (NAP) group. *Tetrahedron Lett.*, **41**, 169–173.

Xia, J., Alderfer, J.L., Piskorz, C.F., and Matta, K.L. (2001) The 2-naphthylmethyl (NAP) group in carbohydrate synthesis: first total synthesis of the GlyCAM-1 oligosaccharide structures. *Chem. Eur. J.*, 356–367.

Xia, Y., Vetvicka, V., Yan, J., Hanikyrová, M., Mayadas, T.N., and Ross, G.D. (1999) The β-glucan-binding lectin site of mouse CR3 (CD11b/CD18) and its function in generating a primed state of the receptor that mediates cytotoxic activation in response to iC3b-opsonized target cells. *J. Immunol.*, **162**, 2281–2290.

Yan, J., Vetvicka, V., Xia, Y., Coxon, A., Carroll, M.C., Mayadas, T.N., and Ross, G.D. (1999) β-Glucan, a "specific" biologic rsponse modifier that uses antibodies to target tumors for cytotoxic recognition by leukocyte complement receptor type 3 (CD11b/CD18). *J. Immunol.*, **163**, 3045–3052.

Yan, J., Zong, H., Shen, A., Chen, S., Yin, X., Shen, X., Liu, W., Gu, X., and Gu, J. (2003) The β-(1,6)-branched β-(1,3) glucohexaose and its analogues containing an α-(1,3)-linked bond have similar stimulatory effects on the mouse spleen as lentinan. *Int. Immunopharmacol.*, **3**, 1861–1871.

Zeng, Y. and Kong, F. (2003) Synthesis of β-D-glucose oligosaccharides from *Phytophthora parasitica*. *Carbohydr. Res.*, **338**, 2359–2366.

Zeng, Y., Zhang, W., Ning, J., and Kong, F. (2002) Synthesis of two isomeric pentasaccharides, the possible repeating unit of the β-glucan from the micro fungus *Epicoccum nigrum* Ehrenb. Ex Schlecht. *Carbohydr. Res.*, **337**, 2382–2391.

Zhao, W., Yang, G., and Kong, F. (2003) Synthesis of two heptasaccharide analogues of the lentinan repeating unit. *Carbohydr. Res.*, **338**, 2813–2823.