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Chemical Synthesis and Application of Biotinylated Oligo- α -(1 $\rightarrow$ 3)-D-Glucosides To Study the Antibody and Cytokine Response against the Cell Wall α -(1 $\rightarrow$ 3)-D-Glucan of *Aspergillus fumigatus*

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Abstract

Biotinylated hepta-, nona- and undeca- α -(1 $\rightarrow$ 3)-D-glucosides representing long oligosaccharides of α -(1 $\rightarrow$ 3)-D-glucan, one of the major components of the cell walls of the fungal pathogen *Aspergillus fumigatus*, were synthesized for the first time via a blockwise strategy. Convergent assembly of the α -(1 $\rightarrow$ 3)-D-glucan chains was achieved by glycosylation with oligoglucoside derivatives bearing 6-*O*-benzoyl groups. Those groups are capable of remote α -stereocontrolling participation, making them efficient α -directing tools even in the case of large glycosyl donors. Synthetic biotinylated oligoglucosides (and biotinylated derivatives of previously synthesized tri- and penta- α -(1 $\rightarrow$ 3)-D-glucosides) loaded on streptavidin microtiter plates were shown to be better recognized by anti- α -(1 $\rightarrow$ 3)-glucan human polyclonal antibodies and to induce higher cytokine responses upon stimulation of human peripheral blood mononuclear cells than their natural counterpart, α -(1 $\rightarrow$ 3)-D-glucan, immobilized on a conventional microtiter plate. Attachment of the synthetic oligosaccharides equipped with a hydrophilic spacer via the streptavidin–biotin pair allows better spatial presentation and control of the loading compared to

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The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

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¹H NMR and ¹³C NMR spectra of compounds 2, 4–6, 8–18, 20, 21, and 22 and immunolabeling of germ tubes of *A. fumigatus* (PDF)

the random sorption of natural α -(1 $\rightarrow$ 3)-glucan. Increase of oligoglucoside length results in their better recognition and enhancement of cytokine production. Thus, using synthetic α -(1 $\rightarrow$ 3)-glucan oligosaccharides, we developed an assay for the host immune response that is more sensitive than the assay based on native α -(1 $\rightarrow$ 3)-glucan.

Graphical Abstract

INTRODUCTION

Aspergillus fumigatus is the most ubiquitous airborne human fungal pathogen.¹ The fungal cell wall is at the forefront of interactions between the host and pathogen and thus requires extensive investigation. The cell wall of *A. fumigatus* is a complex and dynamic structure, and it is mainly composed of polysaccharides such as β -(1 $\rightarrow$ 3)-D-glucan, α -(1 $\rightarrow$ 3)-D-glucan, chitin, galactomannan and galactosaminogalactan.² α -(1 $\rightarrow$ 3)-Glucan is the major amorphous component of the *A. fumigatus* cell wall (accounting for approximately 20 and 40% of the conidium and mycelium cell walls, respectively).³ It was shown in murine models of aspergillosis that α -(1 $\rightarrow$ 3)glucan plays a role in innate and adaptive immunity.^{4,5} However, the high molecular weight and water insolubility of α -(1 $\rightarrow$ 3)-glucan restrict its potential applications. Thus, synthetic α -(1 $\rightarrow$ 3)-glucan oligosaccharides with different degrees of polymerization (dp values) will be invaluable tools for analyzing the host immune response against α -(1 $\rightarrow$ 3)-glucan,⁶ especially since the size of the α -(1 $\rightarrow$ 3)-glucan molecule can be used to tune the immune response.⁷

We describe here the preparation of spaced hepta-, nona- and undeca- α -(1 $\rightarrow$ 3)-D-glucosides. During their synthesis, we also studied the applicability of methods used for controlling α -stereoselectivity in case of small glycosyl donors for glucosylations with much larger donors. The corresponding biotinylated derivatives of these oligosaccharides and previously synthesized⁶ tri- and pentaglucoside derivatives were also prepared. These oligosaccharides of different chain lengths were used to analyze the immune response in comparison to that of the native α -(1 $\rightarrow$ 3)-glucan extracted from the *A. fumigatus* cell wall.

RESULTS AND DISCUSSION

Synthesis of Hepta-, Nona- and Undecasaccharides Related to α -(1 $\rightarrow$ 3)-Glucan of *A. fumigatus* and Their Biotinylated Conjugates.

The synthesis of the target compounds, representing large oligosaccharides, was achieved by a convergent scheme based on the use of a tetrasaccharide glycosyl donor block (Scheme 1). The main problem in the assembly of oligoglucoside chains of this type is the 1,2-*cis* stereoselectivity in the synthesis of α -glucosides, which is known to be a nontrivial task.⁸ To develop new methods for 1,2-*cis*-selective glycosylations, we have been constantly investigating the effect of stereoelectronic properties and other electronic interactions of protecting groups on the stereochemistry of the glycosylation,^{9–14} and in particular, we have studied the possibility of remote anchimeric assistance. To achieve this goal, the use of the corresponding 6-*O*-benzoylated glycosylating agents was explored. The benzoyl group at O-6 is thought to possess an α -stereodirecting effect because of its ability to shield the β -face of the reaction intermediate, an oxocarbenium ion, the structure of which controls the stereochemistry of the glycosylation.^{6,10,11,14}

We successfully employed a stereoselective α -glucosylation for the assembly of short α -glucooligosaccharide chains using mono- and disaccharide glucosyl donors.^{6,10,14,15} Thus, we investigated the applicability of the discussed methods for achieving stereocontrolled α -glucosylations with large oligosaccharide glycosyl donors.

The synthesis of the tetrasaccharide donor bearing a stereodirecting benzoyl group at O-6 started from the transformation of disaccharide block **1** into disaccharide acceptor **2** by treatment with hydrazine acetate. The tetrasaccharide chain was built by the α -selective coupling of disaccharide donor **3** and acceptor **2** assisted by the α -directing benzoyl group at O-6 of the donor. After a routine purification, α -linked tetrasaccharide product **4** was isolated in 79% yield along with a minor amount of the β -linked byproduct (Scheme 1).

On the way to tetrasaccharide donor **6**, glycoside **4** was converted into hemiacetal **5** in 74% yield by removing the *p*-methoxyphenyl group with CAN in a heterogeneous mixture of CH₃CN, benzene and water.⁶ Then, hemiacetal **5** was converted to trifluoroacetimidate donor **6** by treatment with N-phenyltrifluoroacetimidoyl chloride in the presence of K₂CO₃. The reaction of tetrasaccharide donor **6** with previously synthesized⁶ acceptor **7** afforded stereochemically pure heptasaccharide **8** in 63% yield (based on donor **6**) after HPLC purification. The optimum order for mixing the reagents was found to be the simultaneous addition of donor **6** and the promoter to acceptor **7**, which was used in a two-fold excess and had been pretreated with activated molecular sieves. At the end of the reaction, the excess acceptor **7** was fully recovered.

Compound **8** was transformed into heptasaccharide acceptor **9** by removal of the levulinoyl group with hydrazine acetate. Glycosylation of the acceptor with 2 equiv of 6-*O*-benzoylated disaccharide donor **3** produced nonasaccharide **10** in 45% yield. Considering that the reversed order of addition of the reagents increased the yield of heptasaccharide **8**, the applicability of this trick was explored to increase the yield of nonasaccharide **10**. However, in this case, the reversed order of addition did not change the yield. In addition, the use of 2

equiv of costly acceptor **9** is not practical. Undecasaccharide **11** was prepared by glycosylation of acceptor **9** with 2 equiv of tetrasaccharide donor **6** in 40% yield.

The stereochemistry of the newly formed bonds in hepta-, nona- and undecasaccharides **8**, **10** and **11** and in tetrasaccharide **4** was confirmed by ^1H NMR spectroscopy. The $J_{1,2}$ coupling constants were in the range of 3.5–3.7 Hz, confirming the 1,2-*cis*-orientation of all glucosidic bonds and, in particular, the newly formed bonds. In addition, the chemical shifts of C-1 of each glucose residue (except that at the reducing end) were found to be in the range of δ 96–98 ppm, which is characteristic of carbons in α -glucosidic bonds. Whenever it was not possible to measure the $J_{1,2}$ constants, the HSQC correlations of H-1 and their C-1 counterpart indicated that all C-1 resonances were within δ 96–98 ppm. The full assignment of every ring signal was made for heptasaccharide **8** with the help of its HMBC spectrum, which additionally confirmed the α -configuration of every glycosidic bond in compound **8**.

To prepare free heptasaccharide **12**, compound **9** was initially subjected to hydrogenolysis over $\text{Pd}(\text{OH})_2/\text{C}$ in a mixture of ethyl acetate and methanol. Then, the debenzylated product was treated with NaOH in MeOH to remove the *O*- and *N*-acyl protecting groups. Product **12** was isolated from the reaction mixture in 51% yield by size-exclusion chromatography (Scheme 2).

Due to the poor solubility of partially debenzylated intermediates of **14** and their adhesion to the Pd catalyst, we had to use sodium in liquid ammonia for the removal of the benzyl groups. Protected undecasaccharide **14** was treated with 1 M NaOH to remove the benzoyl groups. Then, a THF solution of **16** was treated with a solution of sodium in liquid ammonia. Compound **18** was obtained in 57% yield after purification by successive size-exclusion and C18 reversed-phase chromatography steps.

Applying the same procedure to nonasaccharide **13** (prepared from **10** by removal of the Lev group) led to free **17** in a high yield (73%). Notably, this yield exceeds that of the hydrogenolysis-debenzoylation sequence used for the synthesis of heptasaccharide **12**.

The structures of compounds **12**, **17** and **18** were confirmed by NMR and HRMS data. The absence of signals corresponding to aryl protons in the ^1H NMR spectra corroborated the removal of benzyl and benzoyl groups, and the integrals of the signals belonging to the anomeric protons at δ 5.41–5.35 and 4.96 confirmed that products **12**, **17** and **18** contained 7, 9 and 11 α -linked monosaccharides, respectively. The signals corresponding to the spacer methylene fragment linked to the nitrogen shifted from δ >3.40 to <3.24, indicating deprotection of the amino group.

Hepta- (**12**), nona- (**17**) and undecasaccharide **18** were conjugated with biotinylated linker **19**¹⁶ to give corresponding biotinylated derivatives **20–22**. The presence of biotin in the products was confirmed by MS and ^1H NMR analyses.

Detection of Anti- α -(1 $\rightarrow$ 3)-Glucan Antibodies in Sera of Patients with *Aspergillus* Infections Using α -(1 $\rightarrow$ 3)-Glucan-Related Oligosaccharides.

Synthetic α -(1 $\rightarrow$ 3)-oligo glucosides **20–22** together with previously synthesized⁶ tri- and pentagluconide derivatives **23** and **24** were used to investigate the presence of anti- α -(1 $\rightarrow$ 3)-glucan-specific antibodies in sera from patients with allergic bronchopulmonary aspergillosis (ABPA) or chronic pulmonary aspergillosis (CPA). Biotinylated α -(1 $\rightarrow$ 3)-oligosaccharides were loaded on streptavidin microtiter plates, and the antibody titers against them, as well as against the natural α -(1 $\rightarrow$ 3)-glucan loaded on a microtiter plate, were evaluated. All biotinylated α -(1 $\rightarrow$ 3)-oligoglucosides **20–24** and the natural α -(1 $\rightarrow$ 3)-glucan were able to detect anti- α -(1 $\rightarrow$ 3)-glucan-specific antibodies in the serum samples (Figure 1).

The levels of α -(1 $\rightarrow$ 3)-glucan-specific antibodies in the sera of the immunocompetent aspergillosis patients (ABPA and CPA) were significantly higher than those in the sera of the control individuals. Moreover, the antibody titer values for the sera of ABPA and CPA patients were similar to those of the penta- to undecaoligosaccharides, while those obtained with the trisaccharide and the natural α -(1 $\rightarrow$ 3)-glucan were lower. Furthermore, the patients with invasive pulmonary aspergillosis (IPA) did not have antibodies against α -(1 $\rightarrow$ 3)-glucan due to suppression of their immune systems (data not shown).

The oligo- α -(1 $\rightarrow$ 3)-glucosides were also used to investigate the epitope recognized by two monoclonal antibodies (MAbs) against α -(1 $\rightarrow$ 3)-glucan, A16 and J558.¹⁷ MAbs A16 and J558 were positively labeled on the surface of the *A. fumigatus* germ tubes (see Figure S1 in the Supporting Information). In contrast, the germ tubes of the triple α -(1 $\rightarrow$ 3)-glucan synthase mutant (*ags1/ags2/ags3*), which does not produce α -(1 $\rightarrow$ 3)-glucan,⁴ were not labeled with these two monoclonal antibodies (Figure S1). By ELISA, we showed that A16 and J558 were also able to recognize the oligo- α -(1 $\rightarrow$ 3)-glucosides from 5 to 11 dp (Figure 2). The slightly lower OD observed with the trisaccharide suggested that the epitope that is recognized by these monoclonal antibodies is linear and at least a tetra- or pentasaccharide. However, none of the monoclonal antibodies could recognize the natural α -(1 $\rightarrow$ 3)-glucan when it was loaded on the microtiter plate, but they were able to react with the mycelial surface and recognize α -(1 $\rightarrow$ 3)-glucan in solution (data not shown). This difference could be explained by an unfavorable spatial presentation of this polymer when loaded on the plastic surface of the microtiter plate. The major conclusion of these experiments is that α -(1 $\rightarrow$ 3)-oligoglucosides can be used to diagnose *Aspergillus* infection (ABPA and CPA) in immunocompetent patients.

Ability of Synthetic α -(1 $\rightarrow$ 3)-Glucan-Related Oligosaccharides To Induce Cytokines.

Peripheral blood mononuclear cells (PBMCs) isolated from human blood samples were stimulated by oligo- α -(1 $\rightarrow$ 3)-glucosides **20–24** that were preimmobilized on streptavidin plates or free in solution. The immunogenic effects of the preimmobilized oligosaccharides were compared to that of the natural α -(1 $\rightarrow$ 3)-glucan in suspension. The production of pro-inflammatory (TNF- α , IL-6 and IL-1 β) and anti-inflammatory cytokines (IL-1Ra) by the PBMCs was then examined and compared with the control (unstimulated PBMCs). Under these experimental conditions, it was found that only the immobilized nona- and

undecasaccharides, **21** and **22**, were able to stimulate cytokine production in PBMCs (Figure 3A–D). In addition, the cytokine induction level was directly proportional to the length of the oligo- α -(1 $\rightarrow$ 3)-glucosides (Figure 3A–D).

Upon immobilization and incubation with PBMCs, the biotinylated nona- and undecasaccharides, **21** and **22**, induced significantly higher cytokine production compared to that in the unstimulated control, while no significant induction was observed with shorter oligo- α -(1 $\rightarrow$ 3)-glucosides **20**, **24** and **23** (although there was a trend in stimulating the production of cytokines by heptasaccharide **20**). Under these experimental conditions, the free natural cell wall α -(1 $\rightarrow$ 3)-glucan was capable of inducing only low levels of pro-inflammatory cytokines (the levels were not significantly different from those in the controls), but a significant induction of the anti-inflammatory cytokine IL-1Ra was observed (Figure 3D). In contrast, microtiter-plate-loaded natural α -(1 $\rightarrow$ 3)-glucan and free oligosaccharides failed to stimulate cytokine secretion in PBMCs (data not shown).

However, the ELISA and cytokine data obtained with biotinylated oligosaccharides **20–24** and natural cell wall α -(1 $\rightarrow$ 3)-glucan cannot be directly compared since very different protocols for immobilization on the microtiter plates were used for these compounds (the biotinylated and natural oligosaccharides). Despite these discrepancies, our results indicated that the spatial presentation of the oligosaccharides on the streptavidin plates is favorable for analysis of the immune response to cell wall polysaccharides.

Similar observations also apply to other immunological studies on cell wall polysaccharides, which demonstrated that shorter soluble oligosaccharides in their free form were not able to elicit significant immune responses despite associating with their respective pattern recognition receptors (PRRs).^{7,18–21}

All these examples are consistent with the “fibril hypothesis”, which proposes that short soluble oligosaccharides can bind to a single pattern-recognition receptor without inducing an immune response, whereas fibrillar polysaccharides can be sensed by several PRR molecules, leading to increased triggering of signaling events and activation of the immune response.^{22,23} Another explanation is that the water-insoluble polysaccharide is phagocytosed and degraded to small fragments in the phagolysosome by reactive oxidants. This random degradation will lead to the production of oligosaccharides of different sizes (as shown for bacterial polysaccharides),²⁴ which can then stimulate an immune response. However, plate-loaded natural α -(1 $\rightarrow$ 3)-glucan failed to induce cytokine secretion in PBMCs, which could be explained by the loss of epitopic signature upon loading.

SUMMARY AND OUTLOOK

In conclusion, hepta-, nona- and undecasaccharides representing larger fragments of α -(1 $\rightarrow$ 3)-glucan were prepared for the first time. Highly α -selective oligosaccharide chain assembly was achieved using a tetrasaccharide glycosyl donor bearing a benzoyl group at O-6, which controls α -bond formation due to remote stereodirecting assistance. The corresponding biotinylated derivatives of the oligo- α -(1 $\rightarrow$ 3)-D-glucosides represent a set

of oligosaccharides that could be used to investigate glycan–protein interactions and cytokine induction associated with the immune response to *A. fumigatus*.

The obtained immunological results showed that synthetic oligo- α -(1 $\rightarrow$ 3)-glucosides are specifically recognized by aspergillosis patient antibodies and are able to induce the production of pro- and anti-inflammatory cytokines. The length of the synthetic oligo- α -(1 $\rightarrow$ 3)-glucoside influences the immune response. The immune response against synthesized oligoglucosides was studied with the help of their biotinylated derivatives immobilized on streptavidin-coated plate. Oligosaccharide recognition using such a protocol is more efficient than using a natural cell wall α -(1 $\rightarrow$ 3)-glucan, whose drawbacks are high molecular weight and water insolubility. Oligoglucosides attached to the microtiter well surface via a streptavidin–biotin pair revealed the critical importance of controlled immobilization and spatial presentation of these ligands for efficient immune response.

Finally, these biotinylated reagents will be invaluable in (1) the development of diagnostic tools for aspergillosis, in distinguishing immunocompetent and immunocompromised patients with aspergillosis and in (2) improving our understanding of the interactions of α -(1 $\rightarrow$ 3)-glucan with host immune systems, especially the spatial epitope structure of α -(1 $\rightarrow$ 3)-glucan in eliciting an immune response, and these results should present new perspectives for the identification and structural assessment of host immune cell receptor(s) for α -(1 $\rightarrow$ 3)-glucan.

EXPERIMENTAL SECTION

Chemical Synthesis of Biotinylated Oligosaccharides 20–22: General Methods.

All glycosylation reactions were carried out under dry Ar. Molecular sieves MS AW-300 for glycosylation reactions were activated prior to use at 180 °C under a vacuum generated by an oil pump for 1 h. Dichloromethane was successively distilled from diethanolamine, P₂O₅, and CaH₂ under Ar. For the glycosylation reactions, dichloromethane was freshly redistilled from CaH₂. Pyridine was dried by distillation from P₂O₅. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F254 aluminum sheets (Merck), and visualization was accomplished using UV light or by charring at 150 °C with 10% (v/v) H₃PO₄ in ethanol. Column chromatography was performed on silica gel 60, 40–63 μ m (Merck). Preparative HPLC separations were performed on a Supelcosil LC-SI column (5 μ m, 250 $\times$ 21.2 mm) at a flow rate of 8 mL/min with a K-2401 refractive index detector (Knauer). Data processing was performed using MultiChrom Software. Optical rotation values were measured using a JASCO DIP-360 polarimeter at ambient temperature in the specified solvent. ¹H and ¹³C NMR spectra were recorded on Bruker AV-400, Bruker AV-600, and Bruker Avance spectrometers. Chemical shifts were referenced to residual solvent signals. Signals in the ¹H and ¹³C NMR spectra were assigned using COSY, TOCSY, and ¹H–¹³C HSQC techniques. High-resolution mass spectra were acquired by electrospray ionization on a Micro-TOF II (Bruker Daltonics) instrument.

p-Methoxyphenyl 6-*O*-benzoyl-2,4-di-*O*-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-benzoyl-2,4-di-*O*-benzyl- β -D-glucopyranoside (**2**). Disaccharide **1** (150 mg, 0.135 mmol) was dissolved in 1.2 mL of a 1 M solution of hydrazine hydrate in pyridine–acetic acid (3:2).

The mixture was stirred for 2 h, then acetylacetone (240 μL , 2.4 mmol) was added, and then the solvents were removed under reduced pressure. Product **2** (134 mg, 98%) was purified by column chromatography (toluene–acetonitrile 20:1). Foam, R_f 0.44 (light petroleum/EtOAc 2:1); $[\alpha]_D^{22} +45.2$ ($c = 1$ in CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ_{H} 8.11 (d, 2H, α -Bz), 8.06 (d, 2H, α -Bz), 7.67 (t, 1H, Ar), 7.60–7.50 (m, 3H, Ar), 7.46–7.18 (m, 22H, Ar), 7.04 (d, $J_{9,1}$ 1 Hz, 2H, $\text{C}_6\text{H}_4\text{OMe}$), 6.78 (d, $J_{9,1}$ 1 Hz, 2H, $\text{C}_6\text{H}_4\text{OMe}$), 5.67 (d, $J_{1^b,2^b}$ 3.7 Hz, 1H, H-1^b), 5.13 (d, J_{gem} 10.1 Hz, 1H, PhCH_2), 5.06 (d, J_{gem} 11.3 Hz, 1H, PhCH_2), 4.97 (d, $J_{1^a,2^a}$ 7.5 Hz, 1H, H-1^a), 4.94 (d, J_{gem} 11.1 Hz, 1H, PhCH_2), 4.81 (d, J_{gem} 10.1, 1H, PhCH_2), 4.74 (d, J_{gem} 11.8 Hz, 1H, PhCH_2), 4.72–4.69 (m, 2H, H-6A^a, PhCH_2), 4.66–4.59 (m, 2H, PhCH_2), 4.56 (m, 1H, H-5^b), 4.45–4.38 (m, 2H, H-6B^a, H-6A^b), 4.30 (t, $J_{3^b,2^b} = J_{3^b,4^b}$ 9.7 Hz, 1H, H-3^b), 4.22 (dd, $J_{6\text{Bb},5\text{b}}$ 4.6 Hz, $J_{6\text{B}^b,6\text{B}^a}$ 12.1 Hz, 1H, H-6B^b), 4.14 (t, $J_{3^a,2^a} = J_{3^a,4^a}$ 8.6 Hz, H-3^a), 3.89–3.83 (m, 3H, H-5^a, H-2^a, H-4^a), 3.82 (s, 3H, OCH_3), 3.56 (t, $J_{4\text{b},3\text{b}} = J_{4\text{b},5\text{b}}$ 9.8 Hz, 1H, H-4b), 3.49 (dd, $J_{2\text{b},1\text{b}}$ 3.6 Hz, $J_{2\text{b},3\text{b}}$ 9.8 Hz, 1H, H-2^b); ^{13}C NMR (150.9 MHz, CDCl_3) δ_{C} 166.1 (PhC(O)), 155.4 ($\text{C}_6\text{H}_4\text{OCH}_3$), 151.3 ($\text{C}_6\text{H}_4\text{OCH}_3$), 137.5, 133.1, 132.9, 130.1, 129.7, 128.7, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.3 (Ar), 118.3, 114.5 ($\text{C}_6\text{H}_4\text{OCH}_3$), 102.9 (C-1^a), 96.6 (C-1^b), 79.9 (C-2^a), 79.6, 79.5 (C-3^a, C-2^b), 78.9 (C-4^a), 78.1 (C-4^b), 74.9, 74.7, 74.0 (PhCH_2), 73.9 (C-3^b), 73.5 (PhCH_2), 72.9 (C-5^a), 68.8 (C-5^b), 63.5 (C-6^a, C-6^b), 55.6 (OCH_3). Anal. calcd for $\text{C}_{61}\text{H}_{60}\text{O}_{14}$: C, 72.03; H, 5.95. Found: C, 71.81; H, 5.80.

p-Methoxyphenyl 6-*O*-benzoyl-2,4-di-*O*-benzyl-3-*O*-levulinoyl- α -*D*-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-benzoyl-2,4-di-*O*-benzyl- α -*D*-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-benzoyl-2,4-di-*O*-benzyl- β -*D*-glucopyranoside (**4**). A solution of donor **3** (318 mg, 0.270 mmol) and acceptor **2** (229 mg, 0.225 mmol) in dry CH_2Cl_2 (5.5 mL) was stirred with MS AW 300 (550 mg) for 1 h. Then, the mixture was cooled to -35°C , and MeOTf (30.5 μL , 0.270 mmol) was added. The solution was allowed to warm slowly to -10°C . After 2 h, methanol (30 μL) and triethylamine (60 μL) were added. The solids were removed by filtration and washed with CH_2Cl_2 , and the filtrate was washed with saturated aqueous NaHCO_3 and water. The organic layer was separated, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (light petroleum/EtOAc 2:1) and by HPLC (toluene/acetonitrile 15:1) to afford foam **4** (355 mg, 0.178 mmol, 79%) and **4 β** (13 mg, 0.0065 mmol, 3%).

4: Foam, R_f 0.17 (light petroleum/EtOAc 2:1); $[\alpha]_D^{26} +103.0$ ($c = 1$ in CHCl_3); ^1H NMR (600 MHz, CDCl_3) δ_{H} 8.13–8.05 (m, 8H, α -Bz), 7.66–6.98 (m, 54H, Ar), 6.76 (d, $J_{9,1}$ 1 Hz, 2H, $\text{C}_6\text{H}_4\text{OMe}$), 5.78 (d, $J_{1^c,2^c}$ 3.6 Hz, 1H, H-1^c), 5.71 (t, $J_{3^d,2^d} = J_{3^d,4^d}$ 9.6 Hz, 1H, H-3^d), 5.66 (d, $J_{1^d,2^d}$ 3.5 Hz, 1H, H-1^d), 5.64 (d, $J_{1^b,2^b}$ 3.7 Hz, 1H, H-1^b), 5.08 (d, J_{gem} 10.5 Hz 1H, PhCH_2), 4.92 (d, $J_{1^a,2^a}$ 7.4 Hz, 1H, H-1^a), 4.90–4.82 (m, 4H, 4 PhCH_2), 4.71–4.67 (m, 3H, 2 PhCH_2 , H-6A^a), 4.63 (m, 1H, H-5^b), 4.57–4.47 (m, 10H, H-5^c, H-5^d, H-3^b, H-6A^b, 7 PhCH_2), 4.46–4.35 (m, 6H, H-6A^c, H-6A^d, H-6B^a, H-3^c, 2 PhCH_2), 4.19–4.14 (m, 2H, H-3^a, H-6B^b), 4.10–4.04 (m, 7H, H-6B^c, H-6B^d), 3.88–3.81 (m, 4H, H-4^a, H-2^a, H-5^a, H-4^c), 3.80 (s, 1H, OCH_3), 3.76 (t, $J_{4^b,3^b} = J_{4^b,5^b}$ 9.7 Hz, 1H, H-4^b), 3.63–3.54 (m, 3H, H-2^b, H-2^c, H-4^d), 3.50 (dd, $J_{2^d,1^d}$ 3.5 Hz, $J_{2^d,3^d}$ 10.1 Hz, 1H, H-2^c), 2.66–2.63 (m, 2H, $\text{CH}_2\text{CH}_2\text{C(O)CH}_3$), 2.47–2.43 (m, 2H, $\text{CH}_2\text{CH}_2\text{C(O)CH}_3$), 2.17 (s, 3H, CH_3); ^{13}C NMR (150.9 MHz, CDCl_3) δ_{C} 206.1 ($\text{CH}_3\text{C(O)CH}_2$), 171.8 ($\text{CH}_2\text{C(O)O}$), 166.1 (PhC(O)), 155.3

(C₆H₄OCH₃), 151.3 (C₆H₄OCH₃), 137.7, 137.6, 137.5, 137.4, 133.0, 130.2, 130.1, 129.7, 128.5, 128.4, 128.3, 128.2, 128.1, 127.7, 127.6, 127.5, 127.3, 127.1, 126.7, 126.6 (Ar), 118.3, 114.5 (C₆H₄OCH₃), 102.8 (C-1^a), 96.9 (C-1^d), 95.9, 95.8 (C-1^b, C-1^c), 79.72, 79.66 (C-2^a, C-4^b), 79.2 (C-3^a, C-4^c), 78.83, 78.75, 78.61 (C-4^a, C-2^c, C-2^b), 77.7 (C-2^d), 76.5 (C-4^d), 76.1 (C-3^c), 75.4 (C-3^b), 74.3 (PhCH₂), 74.1 (C-3^b, PhCH₂), 73.4, 73.3, 73.2, 73.1 (PhCH₂), 72.8 (C-5^a), 68.5 (C-5^b, C-5^c, C-5^d), 63.7 (C-6^a), 63.5 (C-6^b), 63.3 (C-6^c), 63.2 (C-6^d), 55.6 (OCH₃), 37.8 (CH₂CH₂C(O)CH₃), 29.8 (CH₃), 28.1 (CH₂CH₂C(O)CH₃). Anal. calcd for C₁₂₀H₁₁₈O₂₈: C, 71.77; H, 5.92. Found: C, 71.73; H, 5.77.

4β: Foam, *R_f* 0.17 (light petroleum/EtOAc 2:1); [α]_D²⁶ +56.8 (*c* = 1 in CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ _H 8.12–8.03 (m, 8H, α -Bz), 7.65–7.10 (m, 52H, Ar), 6.98 (d, *J* 9.1 Hz, 2H, C₆H₄OMe), 6.75 (d, *J* 9.1 Hz, 2H, C₆H₄OMe), 5.78 (t, *J*_{3^d,2^d} = *J*_{3^d,4^d} 9.8 Hz, 1H, H-3^d), 5.63 (d, *J*_{1^d,2^d} 3.5 Hz, 1H, H-1^d), 5.58 (d, *J*_{1^b,2^b} 3.4 Hz, 1H, H-1^b), 5.19 (d, *J*_{1^c,2^c} 7.9 Hz, 1H, H-1^c), 5.12 (d, *J*_{gem} 10.5 Hz, 1H, PhCH₂), 5.05 (d, *J*_{gem} 11.0 Hz, 1H, PhCH₂), 4.99 (d, *J*_{gem} 11.0 Hz, 1H, PhCH₂), 4.94 (d, *J*_{gem} 12.3 Hz, 1H, PhCH₂), 4.92 (d, *J*_{gem} 13.0 Hz, 1H, PhCH₂), 4.89 (d, *J*_{1^a,2^a} 7.6 Hz, 1H, H-1^a), 4.86 (d, *J*_{gem} 11.6 Hz, 1H, PhCH₂), 4.76 (d, *J*_{gem} 9.9 Hz, 1H, PhCH₂), 4.68–4.56 (m, 6H, 4PhCH₂, H-5^d, H-6A^a, H-3^b), 4.55–4.41 (m, 8H, H-5^b, H-6A^b, H-6A^c, 5PhCH₂), 4.40–4.31 (m, 4H, H-A^c, H-6B^a, H-6A^d, H-6B^b), 4.19–4.12 (m, 2H, H-6B^c, H-6B^d), 4.05 (t, *J*_{3^a,2^a} = *J*_{3^a,4^a} 9.6 Hz, 1H, H-3^a), 3.95 (t, *J*_{3^c,3^c} = *J*_{3^c,4^c} 9.0 Hz, 1H, H-3^c), 3.86–3.77 (m, 6H, H-4^c, H-5^a, H-2^a, OCH₃), 3.73–3.68 (m, 1H, H-4^a, H-4^d), 3.62–3.53 (m, 4H, H-2^b, H-2^c, H-4^b, H-2^d), 3.24 (m, 1H, H-5^c), 2.68–2.65 (m, 2H, CH₂CH₂C(O)CH₃), 2.52–2.47 (m, 2H, CH₂CH₂C(O)CH₃), 2.17 (s, 3H, CH₃); ¹³C NMR (150.9 MHz, CDCl₃) δ _C 206.1 (CH₃C(O)-CH₂), 171.8 (CH₂C(O)O), 166.1 (PhC(O)), 155.3 (C₆H₄OCH₃), 151.3 (C₆H₄OCH₃), 137.8, 137.6, 137.5, 137.3, 133.0, 130.1, 129.7, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.4, 126.9 (Ar), 118.2, 114.5 (C₆H₄OCH₃), 102.8 (C-1^a), 102.5 (C-1^c), 96.8 (C-1^d), 96.3 (C-1^b), 81.6, 81.5 (C-2^b, C-2^c), 81.1 (C-3^a), 80.6 (C-2^a), 79.2 (C-4^c), 78.7 (C-3^c), 78.3 (C-4^a), 77.8 (C-2^d), 77.2 (C-3^b), 76.3 (C-4^d), 75.9 (C-4^b, PhCH₂), 75.6, 74.5, 74.2, 73.5, 73.4, 73.1 (C-3^d, PhCH₂), 72.7 (C-5^a), 72.3 (C-5^c), 69.3 (C-5^b), 68.8 (C-5^d), 63.9 (C-6^b), 63.8 (C-6^a), 63.2 (C-6^c), 62.8 (C-6^d), 55.6 (OCH₃), 37.7 (CH₂CH₂C(O)CH₃), 29.7 (CH₃), 28.1 (CH₂CH₂C(O)CH₃). Anal. calcd for C₁₂₀H₁₁₈O₂₈: C, 71.77; H, 5.92. Found: C, 71.65; H, 5.83.

6-O-Benzoyl-2,4-di-O-benzyl-3-O-levulinoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- β -D-glucopyranose (5). CAN (482 mg, 0.879 mmol) was added to a suspension of tetrasaccharide **4** (353 mg, 0.176 mmol) in a mixture of acetonitrile/water/benzene (13.6:3.3:1, 14 mL) at 0 °C. The resulting mixture was stirred at this temperature until TLC showed disappearance of the starting material (20 min). The mixture was diluted with EtOAc and washed with saturated aqueous NaHCO₃ and water. The organic layer was separated, and the solvent was evaporated under reduced pressure. Purification of the residue by column chromatography (toluene/EtOAc 7:1) afforded title compound **5** (249 mg, 74%) as a syrupy mixture of α - and β anomers. Syrup, *R_f* (toluene/EtOAc 5:1) 0.25; ¹H NMR (600 MHz, CDCl₃) δ _H 8.16–8.06 (m, Ar), 7.68–6.96 (m, Ar), 5.78–5.75 (m, H-1^{ca}, H-1^{cb}), 5.70 (t, *J* 9.6 Hz, H-3^{da}, H-3^{db}), 5.67 (d, *J* 3.8 Hz,

H-1^{da}, H-1^{db}), 5.66–5.64 (m, H-1^{bb}, H-1^{ba}), 5.29 (m, J₁^{aa}, J₂^{aa} 3.7 Hz, H-1^{aa}), 4.99 (d, J_{gem} 10.6 Hz, PhCH₂), 4.87 (d, J_{gem} 12.1 Hz, PhCH₂), 4.85–4.79 (m, H-1^{ab}, PhCH₂), 4.79–4.72 (m, PhCH₂), 4.71–4.36 (m, bβcβdβaαaβ PhCH₂, H-5^{ba}, H-5^{ca}, H-5^{da}, H-5^{bb}, H-5^{cb}, H-5^{db}, H-6A^{aa}, H-6A^{ab}, H-6B^{aa}, H-6B^{ab}, H-6A^{ba}, H-6A^{ca}, H-6A^{da}, H-6A^{bb}, H-6A^{cb}, H-6A^{db}, H-3^{aa}, H-3^{ba}, H-3^{ca}, H-3^{bb}, H-3^{cb}), 4.33 (m, H-5^{aa}), 4.24–4.04 (m, H-3^{ab}, H-6B^{ba}, H-6B^{ca}, H-6B^{da}, H-6B^{bb}, H-6B^{cb}, H-6B^{db}), 3.88–3.75 (m, H-4^{aa}, H-4^{ab}, H-4^{ba}, H-4^{bb}, H-4^{ca}, H-4^{cb}, H-5^{ab}), 3.68 (dd, J₂^{aa}, J_{1a}^a 3.6 Hz, J_{2a}^a, J_{3a}^a 9.2 Hz, H-2^{aa}), 3.61–3.53 (m, H-4^{da}, H-4^{db}, H-2^{ab}, H-2^{ba}, H-2^{bb}, H-2^{ca}, H-2^{cb}), 3.47–3.43 (dd, H-2^{da}, H-2^{db}), 2.66–2.60 (m, CH₂CH₂C(O)CH₃), 2.46–2.43 (m, CH₂CH₂C(O)CH₃), 2.17 (s, CH₃); ¹³C NMR (150.9 MHz, CDCl₃) δ_C 206.1 (CH₃C(O)CH₂), 171.8 (CH₂C(O)O), 166.2, 166.1 (PhC(O)), 138.0, 137.8, 137.6, 137.5, 137.4, 136.9, 133.1, 133.0, 130.2, 130.1, 130.0, 129.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 127.7, 127.5, 127.4, 127.3, 127.1, 127.0, 126.7, 126.6, 126.5 (Ar), 97.9 (C-1^{ab}), 96.9, 95.8, 95.7 (C-1), 90.4 (C-1^{aa}), 81.1, 79.9, 79.7, 79.2, 78.8, 78.7, 78.6, 78.5, 78.4, 77.7, 76.5, 76.1 (C-2, C-4, C-3), 75.3, 74.9, 74.6, 74.2, 74.0, 73.3, 73.0, 72.9, 72.7 (C-3, C-5, PhCH₂), 68.5, 68.3, 68.2 (C-5), 63.8, 63.5, 63.4, 63.3 (C-6), 55.6 (OCH₃), 37.8 (CH₂CH₂C(O)CH₃), 29.8 (CH₃), 28.1 (CH₂CH₂C(O)CH₃). Anal. calcd for C₁₁₃H₁₁₂O₂₇: C, 71.35; H, 5.94. Found: C, 71.22; H, 5.83.

O-(6-*O*-benzoyl-2,4-di-*O*-benzyl-3-*O*-levulinyl- α -*D*-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-benzoyl-2,4-di-*O*-benzyl- α -*D*-glucopyranosyl-(1 $\rightarrow$ 3)-6-*O*-benzoyl-2,4-di-*O*-benzyl- β -*D*-glucopyranosyl) *N*-phenyltrifluoroacetimidate (**6**). *N*-Phenyltrifluoroacetimidoyl chloride (16.2 μ L, 0.10 mmol) and K₂CO₃ (22 mg, 0.15 mmol) were added to a solution of hemiacetal **6** (128 mg, 0.067 mmol) in acetone (1.3 mL). The reaction mixture was vigorously stirred until consumption of the starting material (monitored by TLC). The mixture was filtered through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was subjected to column chromatography (toluene/EtOAc 12:1) to provide **6** (132 mg, 95%) as a syrupy mixture of α - and β -anomers. Syrup, *R*_f 0.41 (light petroleum/EtOAc 1.5:1); ¹H NMR (600 MHz, CDCl₃) δ _H 8.14–8.06 (m, α -Bz), 7.66–7.01 (m, Ar), 6.80 (d, NPh), 6.71 (d, NPh), 6.49 (bs, 1H, H-1^{aa}), 5.83 (bs, 1H, H-1^{ab}), 5.79–5.76 (mix. H-1), 5.76–5.70 (mix. H-3^d), 5.68–5.63 (mix. H-1), 5.57 (d, *J* 3.6 Hz, H-1 ^{β}), 4.93–4.61 (PhCH₂, H-5, H-6), 4.60–4.35 (PhCH₂, H-3, H-5, H-6), 4.30–4.10 (H-5^{aa}, H-3^{ab}, H-6), 3.90–3.74 (H-2, H-4), 3.64–3.49 (H-2, H-4), 2.68–2.63 (CH₂CH₂C(O)CH₃), 2.48–2.44 (CH₂CH₂C(O)CH₃), 2.17 (s, CH₃); ¹³C NMR (150.9 MHz, CDCl₃) δ _C 206.0 (CH₃C(O)CH₂), 171.8 (CH₂C(O)O), 166.1 (PhC(O)O), 137.9, 137.8, 137.6, 133.1, 133.0, 130.3, 129.7, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.5, 127.3, 127.2, 127.1, 126.9, 126.8, 124.3, 119.5, 119.3 (Ar), 97.0, 96.2, 95.9, 95.7 (C-1), 92.5 (C-1^{aa}), 79.8, 79.6, 79.4, 79.3, 79.1, 79.0, 78.9, 78.8, 78.4, 78.2, 78.0, 77.8, 76.8 (C-2, C-4), 76.4, 75.7, 75.4, 75.2, 74.5, 74.2, 74.1, 73.5, 73.4, 73.3, 73.2, 73.1, 72.8 (PhCH₂, C-3), 71.0 (C-5^{aa}), 68.7, 68.6, 68.4 (C-5), 63.6, 63.4, 63.3, 63.2 (C-6), 37.8 (CH₂CH₂C(O)CH₃), 29.7 (CH₃), 28.2 (CH₂CH₂C(O)CH₃). Anal. calcd for C₁₂₁H₁₁₆F₃NO₂₇: C, 70.10; H, 5.64. Found: C, 70.22; H, 5.73.

3-Trifluoroacetamidopropyl 6-O-benzoyl-2,4-di-O-benzyl-3-O-levulinoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-

glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (8). A solution of acceptor **7**⁶ (414 mg, 0.295 mmol) in dry CH₂Cl₂ (5 mL) was stirred with MS AW 300 (660 mg) for 1 h. Then, the mixture was cooled to −35 °C, and a solution of **6** (245 mg, 0.118 mmol) in CH₂Cl₂ (1.1 mL) and a solution of MeOTf (13 μL, 0.116 mmol) in CH₂Cl₂ (0.275 mL) were slowly added over 1 h. The solution was allowed to warm slowly to −10 °C, and then methanol (50 μL) and triethylamine (50 μL) were added. The solids were removed by filtration and washed with CH₂Cl₂. The filtrate was washed with saturated aqueous NaHCO₃ and water, the organic layer was separated, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (light petroleum/EtOAc 3:1 → 1:1) and by HPLC (hexane/EtOAc 1.3:1) to afford **8** (245 mg, 0.0745 mmol, 63%) and recover **7** (256 mg, 0.182 mmol). Compound **8**, foam, *R*_f 0.16 (hexane/EtOAc 1.5:1); [*α*]_D²³ +175.3 (*c* = 1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ_H 8.16–8.05 (m, 12H, *α*-Bz), 7.67–7.02 (m, 81H, Ar, NH), 6.94–6.89 (m, 4H, Ar), 6.86 (d, 2H, Ar), 6.25 (d, 2H, Ar), 5.73 (d, 1H, *J*_{1^d,2^d} 3.7 Hz, H-1^d), 5.70 (d, 1H, *J*_{1^c,2^c} 3.7 Hz, H-1^c), 5.66 (t, 1H, *J*_{3^g,4^g} = *J*_{3^g,2^g} 9.6 Hz, H-3^g), 5.63 (d, 1H, *J*_{1^f,2^f} 3.7 Hz, H-1^f), 5.61 (d, 1H, *J*_{1^b,2^b} 3.7 Hz, H-1^b), 5.60 (d, 1H, *J*_{1^e,2^e} 3.7 Hz, H-1^e), 5.58 (d, 1H, *J*_{1^g,2^g} 3.6 Hz, H-1^g), 5.28 (s, 1H, PhCH), 4.87 (d, 1H, *J*_{1^a,2^a} 3.7 Hz, H-1^a), 4.81–4.25 (m, 45H, 26PhCH₂, H-5^{b–g}, H-6A^{a–g}, H-3^{a–f}), 4.07–3.93 (m, 7H, H-6B^{b–g}, OCH₂CH₂CH₂NA), 3.89–3.80 (m, 3H, H-4^b, H-4^c, H-5^a), 3.81–3.69 (m, 7H, H-4^a, H-4^d, H-4^e, H-4^f, H-6B^a, H-2^a, OCH₂CH₂CH₂NA), 3.59–3.40 (m, 9H, H-4^g, 6H-2, OCH₂CH₂CH₂NB, OCH₂CH₂CH₂NB), 2.62 (m, 2H, CH₂CH₂C(O)CH₃), 2.41 (m, 2H, CH₂CHC(O)CH₃), 2.17 (s, 1H, CH₃), 1.99 (m, 2H, OCH₂CH₂CH₂N); ¹³C NMR (100.6 MHz, CDCl₃) δ_C 206.1 (CH₂C(O)CH₃), 171.8 (CH₂C(O)O), 166.3, 166.2 (PhOC(O)), 137.7, 133.0, 130.2, 129.7, 129.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.9, 126.7, 126.5, 126.4, 126.3 (Ar), 102.3 (PhCH), 97.9 (C-1^a), 96.8 (C-1^g), 96.1 (C-1^c), 95.6 (C-1^e), 95.5 (C-1^d, C-1^f), 95.3 (C-1^b), 82.8 (C-4^a), 79.5, 79.4, 79.2 (C-4^{b–f}), 78.4 (C-2^b, C-2^c), 78.2 (C-2^d, C-2^e), 78.1 (C-2^a), 77.6 (C-2^g), 76.8 (C-2^f), 76.5 (C-4^g), 75.9 (C-3^f), 75.2 (C-3^b), 74.5, 74.4 (C-3^c, C-3^d, C-3^e), 74.0 (PhCH₂, C-3^g), 73.6, 73.2, 73.1, 72.9, 72.8, 72.7, 72.6, 72.5, 72.0, 71.9, 71.6, 70.6 (PhCH₂, C-3^a), 69.0 (C-6^a), 68.4, 68.1, 68.0, 67.5 (C-5^{b–g}), 67.2 (OCH₂CH₂CH₂N), 63.6, 63.3, 63.2, 63.0 (C-6^{b–g}), 62.3 (C-5^a), 38.5 (OCH₂CH₂CH₂N), 37.7 (CH₂CH₂C(O)CH₃), 29.8 (CH₃), 28.4 (OCH₂CH₂CH₂N), 28.1 (CH₂CH₂C(O)CH₃). Anal. calcd for C₁₉₂H₁₉₀F₃NO₄₅: C, 70.12; H, 5.82; N, 0.43. Found: C, 70.24; H, 5.96; N, 0.40.

3-Trifluoroacetamidopropyl 6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (9).

Heptasaccharide **8** (29 mg, 0.0069 mmol) was dissolved in 0.5 mL of a 1 M solution of hydrazine hydrate in pyridine–acetic acid (3:2). After 2 h, acetylacetone (102 μL, 1 mmol) was added, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (toluene–EtOAc, 15:1 → 5:1) to give compound **9** (25 mg, 89%) as a foam. *R*_f 0.40 (hexane/EtOAc 1.2:1); [*α*]_D²³ +174.2 (*c* = 1, CHCl₃); ¹H NMR

(600 MHz, CDCl₃) δ_{H} 8.15–8.03 (m, 12H, α -Bz), 7.65–7.00 (m, 77H, Ar, NH), 6.93–6.87 (m, 4H, Ar), 6.84 (d, 2H, Ar), 6.68–6.64 (m, 4H, Ar), 6.22 (d, 2H, Ar), 5.71 (d, 1H, $J_{1^{\text{d}},2^{\text{d}}}$ 3.8 Hz, H-1^d), 5.69 (d, 1H, $J_{1^{\text{c}},2^{\text{c}}}$ 3.7 Hz, H-1^c), 5.60 (d, 2H, H-1^b, H-1^f), 5.58 (d, 1H, $J_{1^{\text{e}},2^{\text{e}}}$ 3.8 Hz, H-1^e), 5.56 (d, 1H, $J_{1^{\text{g}},2^{\text{g}}}$ 3.6 Hz, H-1^g), 5.26 (s, 1H, PhCH), 4.87 (d, 1H, $J_{1^{\text{a}},2^{\text{a}}}$ 3.8 Hz, H-1^a), 4.79–4.23 (m, 45H, 26PhCH₂, H-5^{b-g}, H-6A^{a-g}, H-3^{a-f}), 4.12 (t, $J_{3^{\text{g}},2^{\text{g}}} = J_{3^{\text{g}},4^{\text{g}}}$ 9.3 Hz, 1H, H-3^g), 4.08 (m, 1H, H-6B^g), 4.05–3.91 (m, 6H, H-6B^{b-f}, OCH₂CH₂CH₂NA), 3.90–3.81 (m, 3H, H-4^b, H-4^c, H-5^a), 3.79–3.69 (m, 7H, H-4^a, H-4^d, H-4^e, H-4^f, H-6B^a, H-2^a, OCH₂CH₂CH₂NA), 3.54 (dd, $J_{2^{\text{c}},1^{\text{c}}}$ 3.2 Hz, $J_{2^{\text{c}},3^{\text{c}}}$ 10.3 Hz, 1H, H-2^c), 3.52–3.38 (m, 8H, H-4^g, 5H-2, OCH₂CH₂CH₂NB, OCH₂CH₂CH₂NB), 3.35 (dd, $J_{2^{\text{g}},1^{\text{g}}}$ 3.5 Hz, $J_{2^{\text{g}},3^{\text{g}}}$ 9.8 Hz, H-2^g), 1.98 (m, 2H, OCH₂CH₂CH₂N); ¹³C NMR (150.9 MHz, CDCl₃) δ_{C} 171.8 (CH₂C(O)O), 166.2 (PhOC(O)), 137.7, 137.6, 137.5, 133.1, 130.1, 129.9, 129.6, 129.3, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1, 127.0, 126.8, 126.7, 126.6, 126.5, 126.3, 126.2, 126.1 (Ar), 102.3 (PhCH), 97.8 (C-1^a), 96.6 (C-1^g), 96.0 (C-1^c), 95.5 (C-1^d, C-1^e, C-1^f), 95.2 (C-1^b), 82.7 (C-4^a), 79.4, 79.3, 79.2 (C-4^{b-f}), 79.1 (C-2^g), 78.3, 78.0, 77.8 (C-2^{a-f}), 76.8 (C-4^g), 75.5 (C-3^f), 75.1 (C-3^b), 74.3, 74.2 (C-3^c, C-3^d, C-3^e, PhCH₂), 73.6, 73.4 (PhCH₂, C-3^g), 73.0, 72.8, 72.7, 72.6, 72.5, 72.4, 71.9, 71.8, 71.6 (PhCH₂, C-3^a), 70.5 (PhCH₂), 69.0 (C-6^a), 68.1, 67.9, 67.6, 67.3 (C-5^{b-g}), 67.2 (OCH₂CH₂CH₂N), 63.5, 63.2, 62.9 (C-6^{b-g}), 62.2 (C-5^a), 38.5 (OCH₂CH₂CH₂N), 28.4 (OCH₂CH₂CH₂N). Anal. calcd for C₁₈₇H₁₈₄F₃NO₄₃: C, 70.40; H, 5.81; N, 0.44. Found: C, 70.14; H, 5.94; N, 0.54.

3-Trifluoroacetamidopropyl 6-O-benzoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranoside (12). To a solution of **9** (45 mg, 0.0137 mmol) in methanol (2.8 mL) and EtOAc (0.85 mL) was added Pd(OH)₂/C (45 mg), and the resulting mixture was vigorously stirred under hydrogen overnight. The catalyst was removed by filtration through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was dissolved in MeOH (6 mL), and aqueous 1 M NaOH (0.6 mL) was added. The solution was allowed to react for 12 h and then neutralized with 1 M AcOH (0.6 mL) and concentrated. The residue was subjected to gel-permeation chromatography on a TSK HW-40(S) column in 0.1 M AcOH. The appropriate fractions were pooled and freeze-dried to give **12** (8.5 mg, 51%) as a fluffy solid. R_f 0.14 (n-butanol/ethanol/water/15% NH₄OH 0.5:1:0.8:0.4); $[\alpha]_{\text{D}}^{24} +205.2$ ($c = 1$ in H₂O); ¹H NMR (600 MHz, D₂O) δ_{H} 5.41–5.35 (m, 6H, H-1^{b-g}), 4.96 (d, $J_{1^{\text{a}},2^{\text{a}}}$ 3.8 Hz, 1H, H-1^a), 4.08–4.01 (m, 6H, H-5^{b-g}), 3.95–3.61 (m, 28H, OCH₂CH₂CH₂N, H-3^{a-g}, H-6^{a-g}, H-4^{a-f}, H-2^{a-f}, H-5^a), 3.58 (dd, $J_{2^{\text{g}},1^{\text{g}}}$ 3.9 Hz, $J_{2^{\text{g}},3^{\text{g}}}$ 9.9 Hz, 1H, H-2^g), 3.45 (t, $J_{4^{\text{g}},3^{\text{g}}} = J_{4^{\text{g}},2^{\text{g}}}$ 9.6 Hz, 1H, H-4^g), 3.24–3.12 (m, 2H, 2OCH₂CH₂CH₂N), 2.08–2.00 (m, 2H, 2OCH₂CH₂CH₂N); ¹³C NMR (150.9 MHz, D₂O) δ_{C} 100.6, 100.5 (C-1^{b-g}), 99.8 (C-1^a), 81.5, 81.3, 81.1 (C-3^{a-f}), 74.2 (C-3^g), 73.1, 73.0, 72.9 (C-5^{a-f}, C-2^g), 71.7, 71.0, 70.8 (C-2^{a-f}, C-4^{a-g}), 67.1 (OCH₂CH₂CH₂N), 61.8, 61.5 (C-6^{a-g}), 39.1 (OCH₂CH₂CH₂N), 27.8 (OCH₂CH₂CH₂N); HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₄₅H₈₀NO₃₆ 1210.4455, found 1210.4431.

3-Trifluoroacetamidopropyl 6-O-benzoyl-2,4-di-O-benzyl-3-O-levulinoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-

benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside (10). A solution of donor **3** (54 mg, 0.046 mmol) and heptasaccharide acceptor **9** (80 mg, 0.025 mmol) in dry CH₂Cl₂ (1.3 mL) was stirred with MS AW 300 (130 mg) for 1 h. Then, the mixture was cooled to –35 °C and MeOTf (5.2 μ L, 0.046 mmol) was added. The solution was allowed to warm slowly to –10 °C. After 2 h, methanol (10 μ L) and triethylamine (15 μ L) were added. The solids were removed by filtration and washed with CH₂Cl₂. The filtrate was washed with saturated aqueous NaHCO₃ and water, the organic layer was separated, and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (light petroleum/EtOAc 2:1 $\rightarrow$ 1.5:1) and by HPLC (hexane/EtOAc 1.1:1) to afford **10** (47 mg, 0.011 mmol, 45%) as a foam. *R*_f 0.26 (hexane/EtOAc 1.2:1); [α]_D²⁴ +180.0 (*c* = 1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ _H 8.18–8.06 (m, 16H, α -Bz), 7.68–7.03 (m, 101H, Ar, NH), 6.95–6.85 (m, 6H, Ar), 6.78–6.71 (m, 8H, Ar), 5.75 (d, 1H, *J*_{1,2} 3.7 Hz, H-1), 5.73 (d, 1H, *J*_{1,2} 3.7 Hz, H-1), 5.66 (t, 1H, *J*_{3,4}ⁱ = *J*_{3,2}ⁱ 9.6 Hz, H-3ⁱ), 5.65–5.60 (m, 5H, H-1), 5.59 (d, 1H, *J*_{1,2} 3.5 Hz, H-1), 5.30 (s, 1H, PhCH), 4.90 (d, 1H, *J*_{1,2}^a 3.7 Hz, H-1^a), 4.84–4.27 (m, 58H, 34PhCH₂, 8H-5, 9H-6A, 8H-3), 4.07–3.94 (m, 9H, 8H-6B, OCH₂CH₂CH₂NA), 3.91–3.85 (m, 3H, 2H-4, H-5^a), 3.82–3.73 (m, 9H, 6H-4, H-6B^a, H-2^a, OCH₂CH₂CH₂NA), 3.60–3.41 (m, 11H, H-4^e, 8H-2, OCH₂CH₂CH₂NB, OCH₂CH₂CH₂NB), 2.64 (m, 2H, CH₂CH₂C(O)CH₃), 2.44 (m, 2H, CH₂CH₂C(O)CH₃), 2.17 (s, 1H, CH₃), 2.00 (m, 2H, OCH₂CH₂CH₂N); ¹³C NMR (150.9 MHz, CDCl₃) δ _C 206.2 (CH₂C(O)CH₃), 171.8 (CH₂C(O)O), 166.4, 166.2 (PhOC(O)), 137.7, 133.1, 130.2, 129.7, 129.6, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.2, 127.0, 126.9, 126.8, 126.7, 126.4, 126.3, 126.2 (Ar), 102.4 (PhCH), 97.9 (C-1^a), 96.8 (C-1), 96.1 (C-1), 95.6 (C-1), 95.5 (C-1), 95.3 (C-1), 82.8 (C-4^a), 79.5, 79.4, 79.3, 79.2 (C-4), 78.4, 78.1, 77.6, 77.3, 77.1, 76.9 (C-2), 76.5 (C-4), 75.9, 75.2, 74.4, 74.0 (C-3), 73.6, 73.2, 73.1, 72.8, 72.7 (PhCH₂, C-3), 72.6, 72.5, 72.0, 71.9, 71.6, 70.6 (PhCH₂), 69.0 (C-6^a), 68.3, 68.1, 68.0, 67.7, 67.4 (C-5), 67.3 (OCH₂CH₂CH₂N), 63.6, 63.3, 63.0 (C6), 62.3 (C-5^a), 38.5 (OCH₂CH₂CH₂N), 37.7 (CH₂CH₂C(O)CH₃), 29.8 (CH₃), 28.4 (OCH₂CH₂CH₂N), 28.0 (CH₂CH₂C(O)CH₃). Anal. calcd for C₂₄₆H₂₄₂F₃NO₅₇: C, 70.66; H, 5.83; N, 0.33. Found: C, 70.61; H, 5.77; N, 0.39.

3-Trifluoroacetamidopropyl 6-O-benzoyl-2,4-di-O-benzyl-3-O-levulinoyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-6-O-benzoyl-2,4-di-O-benzyl- α -D-glucopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl-4,6-O-benzylidene- α -D-glucopyranoside (11). A solution of tetrasaccharide donor **6** (119 mg, 0.057 mmol) and heptasaccharide acceptor **9** (86 mg, 0.027 mmol) in dry CH₂Cl₂ (2 mL) was stirred with MS AW 300 (200 mg) for 1 h. Then, the mixture was cooled to –35 °C and MeOTf (6.5 μ L, 0.057 mmol) was added. The

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128.1, 128.0, 127.8, 127.6, 127.5, 127.3, 127.2, 127.1, 127.0, 126.9, 126.8, 126.7, 126.6, 126.5, 126.3 (Ar), 102.3 (PhCH), 97.9 (C-1^a), 96.6 (C-1), 96.1 (C-1), 95.5 (C-1), 95.3 (C-1), 82.8 (C-4^a), 79.5, 79.4, 79.2 (C-4), 78.5, 78.1, 77.9 (C-2), 75.6, 75.2 (C-3), 74.4, 74.3, 73.6, 73.5, 73.4, 73.1, 72.9, 72.8, 72.7, 72.6, 72.5, 71.9, 71.6, 70.6 (PhCH₂, C-3), 69.0 (C-6^a), 68.2, 68.0, 67.7, 67.5 (C-5), 67.2 (OCH₂CH₂CH₂N), 63.6, 63.3, 63.0 (C-6), 62.3 (C-5^a), 38.5 (OCH₂CH₂CH₂N), 28.0 (OCH₂CH₂CH₂N). Anal. calcd for C₂₄₁H₂₃₆F₃NO₅₅: C, 70.89; H, 5.83. Found: C, 71.05; H, 5.99.

3-Trifluoroacetamidopropyl 6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-6-O-benzoyl-2,4-di-O-benzyl-α-D-glucopyranosyl-(1 → 3)-2-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (14). Undecasaccharide **11** (40 mg, 0.0079 mmol) was dissolved in 0.5 mL of a 1 M solution of hydrazine hydrate in pyridine–acetic acid (3:2), and the mixture was allowed to react for 2 h. Acetylacetone (102 μL, 1 mmol) was added, and the solvents were removed under reduced pressure. The residue was purified by column chromatography (toluene–EtOAc, 15:1 → 5:1) to provide **14** (38 mg, 97%) as a foam. *R*_f 0.40 (toluene/EtOAc 5:1); [α]_D²⁴ +197.5 (*c* = 1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ_H 8.19–8.07 (m, 20H, α-Bz), 7.69–7.04 (m, 123H, Ar, NH), 6.97–6.92 (d, 4H, Ar), 6.89–6.87 (d, 2H, Ar), 6.74–6.67 (m, 12H, Ar), 5.75 (d, 1H, *J*_{1,2} 3.7 Hz, H-1), 5.73 (d, 1H, *J*_{1,2} 3.7 Hz, H-1), 5.66–5.60 (m, 7H, H-1), 5.59 (d, *J* 3.5 Hz, H-1), 5.31 (s, 1H, PhCH), 4.91 (d, 1H, *J*_{1^a,2^a} 3.8 Hz, H-1^a), 4.86–4.27 (m, 73H, 42PhCH₂, 10H-5, 11H-6A, 10H-3), 4.17 (t, *J*_{3^k,2^k} = *J*_{3^k,4^k} 9.2 Hz, 1H, H-3^k), 4.11 (m, 1H, H-6B), 4.07–3.94 (m, 10H, 9H-6B, OCH₂CH₂CH₂NA), 3.94–3.87 (m, 3H, 2H-4, H-5^a), 3.83–3.73 (m, 11H, 8H-4, H-6B^a, H-2^a, OCH₂CH₂CH₂NA), 3.59 (dd, *J* 3.8 Hz, *J* 9.7 Hz, H-2), 3.54 (dd, *J* 3.7 Hz, *J* 9.6 Hz, H-2), 3.53–3.42 (m, 10H, H-4^k, 7H-2, OCH₂CH₂CH₂NB, OCH₂CH₂CH₂NB), 3.39 (m, *J* 3.4 Hz, *J* 9.8 Hz, 1H, H-2), 1.96 (m, 2H, OCH₂CH₂CH₂N); ¹³C NMR (150.9 MHz, CDCl₃) δ_C 166.2 (PhOC(O)), 137.6, 137.5, 133.0, 130.1, 129.6, 129.3, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 127.4, 127.2, 127.0, 126.8, 126.7, 126.5, 126.3, 126.1 (Ar), 102.3 (PhCH), 97.8 (C-1^a), 96.6 (C-1), 96.1 (C-1), 95.5 (C-1), 95.3 (C-1), 82.7 (C-4^a), 79.5, 79.2, 78.3, 77.9, 77.8, 76.8 (C-2, C-4), 75.5, 75.1 (C-3), 74.3, 74.2, 73.6, 73.5, 73.4, 73.0, 72.8, 72.7 (PhCH₂, C-3), 72.4, 71.8, 71.6, 70.5 (PhCH₂), 69.0 (C-6^a), 68.1, 67.9, 67.6 (C-5), 67.2 (OCH₂CH₂CH₂N), 63.5, 63.2, 62.9 (C-6), 62.3 (C-5^a), 38.5 (OCH₂CH₂CH₂N), 28.4 (OCH₂CH₂CH₂N). Anal. calcd for C₂₉₅H₂₈₈F₃NO₆₇: C, 71.20; H, 5.83; N, 0.28. Found: C, 71.31; H, 5.75; N, 0.31.

3-Aminopropyl α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranosyl-(1 → 3)-α-D-glucopyranoside (17). Nonasaccharide **13** (27 mg, 6.1 μmol) was dissolved in 3 mL of CH₂Cl₂/MeOH (1:1), and 3 M NaOH (0.3 mL) was added. The mixture was stirred overnight and then quenched with AcOH. The solvents were evaporated, and the residue was

passed through silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 15:1 $\rightarrow$ 5:1). The product was dissolved in 2.0 mL of anhydrous THF and added dropwise to 10 mL of a dark blue solution of Na in liquid ammonia at -78°C . After 45 min, the mixture was quenched with MeOH, and the ammonia was removed with the stream of argon. The mixture was diluted with water, AcOH was added until pH = 7, and the resulting solution was filtered through a nylon filter. The solvents were evaporated, and the residue was subjected to gel-permeation chromatography on a TSK HW-40(S) column in 0.1 M AcOH and then reversed-phase C_{18} column chromatography and freeze-dried to give **17** (6.9 mg, 57.5%) as a fluffy solid. R_f 0.13 (*n*-butanol/ethanol/water/15% NH_4OH 0.5:1:0.8:0.4); $[\alpha]_D^{23} +254.0$ ($c = 1$ in H_2O); ^1H NMR (600 MHz, D_2O) δ_{H} 5.40–5.35 (m, 8H, H-1^{b-i}), 4.96 (d, $J_{1^a,2^a} 3.8$ Hz, 1H, H-1^a), 4.08–4.02 (m, 8H, H-5^{b-i}), 3.95–3.63 (m, 43H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$, H-3a–i, H-6a–i, H-4a–h, H-2a–h, H-5a), 3.58 (dd, $J_{2i,1i} 3.9$ Hz, $J_{2i,3i} 9.9$ Hz, 1H, H-2ⁱ), 3.45 (t, $J_{4^i,3^i} = J_{4^i,2^i} 10.0$ Hz, 1H, H-4ⁱ), 3.24–3.12 (m, 2H, $2\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.08–2.00 (m, 2H, $2\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$); ^{13}C NMR (150.9 MHz, D_2O) δ_{C} 100.6, 100.5 (C-1^{b-i}), 99.8 (C-1^a), 81.3, 81.1 (C-3^{a-h}), 74.2 (C-3ⁱ), 73.1, 73.0, 72.9 (C-5^{a-i}, C-2ⁱ), 71.6, 71.1, 70.9 (C-2^{a-h}, C-4^{a-i}), 67.1 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 61.8, 61.6 (C-6^{a-i}), 39.1 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 27.8 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$); HRMS (ESI-TOF) m/z $[\text{M} + 2\text{H}]^{2+}$ calcd for $\text{C}_{57}\text{H}_{101}\text{NO}_{46}$ 767.7792, found 767.7781.

*3-Aminopropyl α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranosyl-(1 $\rightarrow$ 3)- α -D-glucopyranoside (**18**).*

Undecasaccharide **14** (32 mg, 6.5 μmol) was dissolved in 3.5 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1), and 3 M NaOH (0.35 mL) was added. The mixture was stirred overnight and then quenched with AcOH. The solvents were evaporated, and the residue was passed through silica gel ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 15:1 $\rightarrow$ 5:1). The residue was dissolved in 2.5 mL of anhydrous THF and added dropwise to 15 mL of a dark blue solution of Na in liquid ammonia at -78°C . After 45 min, the mixture was quenched with MeOH, and the ammonia was removed with the stream of argon. The mixture was diluted with water, AcOH was added until pH = 7, and the solution was filtered through a nylon filter. The solvents were evaporated, and the residue was subjected to gel-permeation chromatography on a TSK HW-40(S) column in 0.1 M AcOH and then reversed-phase C_{18} column chromatography and freeze-dried to give **18** (6.9 mg, 57.5%) as a fluffy solid. R_f 0.13 (*n*-butanol/ethanol/water/15% NH_4OH 0.5:1:0.8:0.4); $[\alpha]_D^{24} +223.1$ ($c = 1$ in H_2O); ^1H NMR (600 MHz, D_2O) δ_{H} 5.40–5.35 (m, 10H, H-1^{b-k}), 4.96 (d, $J_{1^a,2^a} 3.8$ Hz, 1H, H-1^a), 4.08–4.00 (m, 10H, H-5^{b-k}), 3.95–3.65 (m, 43H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{NA}$, H-3^{a-k}, H-6^{a-k}, H-4^{a-j}, H-2^{a-j}, H-5^a), 3.63 (m, 1H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{NB}$), 3.58 (dd, $J_{2^k,1^k} 3.9$ Hz, $J_{2^k,3^k} 9.9$ Hz, 1H, H-2^k), 3.45 (t, $J_{4^k,3^k} = J_{4^k,2^k} 10.0$ Hz, 1H, H-4^k), 3.24–3.10 (m, 2H, $2\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 2.06–1.97 (m, 2H, $2\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$); ^{13}C NMR (125.8 MHz, D_2O) δ_{C} 100.5, 100.4 (C-1^{b-k}), 99.6 (C-1^a), 81.1, 80.9 (C-3^{a-j}), 74.0 (C-3^k), 73.0, 72.9, 72.8 (C-5^{a-k}, C-2^k), 71.5, 71.0, 70.8 (C-2^{a-j}, C-4^{a-j}), 70.6 (C-4^k), 67.0 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 61.6, 61.4 (C-6^{a-k}), 39.0 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$), 27.8 ($\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}$); HRMS (ESI-TOF) m/z $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{69}\text{H}_{120}\text{NO}_{56}$ 1858.6567, found 1858.6555.

*Conjugate of the Pentasaccharide with Biotin (**20**).* A 0.062 M solution of biotin-activated ester **19** in DMF (100 μL) and dry triethylamine (8 μL) were added to a solution of **12** (1.8

mg, 0.0015 mmol) in DMF (100 μ L) + H₂O (100 μ L). After 19 h, the solvent was removed under reduced pressure, and the product was purified by gel-permeation chromatography (TSK HW-40(S), 0.1 M AcOH). The appropriate fractions were freeze-dried to give **20** (1.3 mg, 50%) as a fluffy solid. R_f 0.65 (n-butanol/ethanol/water/15% NH₄OH 0.5:1:0.8:0.4); ¹H NMR (600 MHz, D₂O) δ_H 5.40–5.36 (m, 6H, H-1^{b-g}), 4.92 (d, 1H, $J_{1^a,2^a}$ 3.8 Hz, H-1^a), 4.63 (dd, J 4.9 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.43 (dd, J 4.5 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.08–4.01 (m, 7H, H-5^{a-h}), 3.94–3.88 (m, 5H, H-3^{b-f}), 3.88–3.82 (m, 8H, H-3^a, H-6A^{a-g}), 3.81–3.73 (m, 12H, H-6B^{a-g}, H-3^g, NC(O)CH₂CH₂O, OCH₂CH₂CH₂N^A), 3.72–3.61 (m, 35H, H-4^{a-f}, H-2^{a-f}, H-5^a, 5 OCH₂CH₂O, OCH₂CH₂N), 3.60–3.53 (m, 2H, H-2^g, OCH₂CH₂CH₂N^B), 3.44 (t, $J_{4^g,3^g} = J_{4^g,2^g}$ 9.6 Hz, 1H, H-4^g), 3.40 (t, J 5.4 Hz, 2H, OCH₂CH₂N), 3.37–3.30 (m, 3H, OCH₂CH₂CH₂N, SCH(CH₂)CHNH), 3.00 (dd, J 5.0 Hz, J 13.0 Hz, 1H, SCH(CH₂)CHCHCH₂^A), 2.80 (d, J 13.0 Hz, 1H, SCH(CH₂)-CHCHCH₂^B), 2.53 (t, J 6.2 Hz, 2H, NC(O)CH₂CH₂O), 2.29 (t, J 7.3 Hz, 2H, C(O)CH₂CH₂CH₂CH₂), 1.87 (m, 2H, OCH₂CH₂CH₂N), 1.75 (m, 1H, C(O)CH₂CH₂CH₂CH₂^A), 1.70–1.55 (m, 3H, C(O)CH₂CH₂CH₂CH₂, C(O)CH₂CH₂CH₂CH₂^A), 1.46–1.39 (m, 2H, C(O)CH₂CH₂CH₂CH₂); ¹³C NMR (150.9 MHz, D₂O) δ_C 177.7, 174.7 (C(O)), 100.6, 100.4 (C-1^{b-g}), 99.5 (C-1^a), 81.4, 81.3 (C-3^{a-f}), 74.1 (C-3^g), 73.1, 73.0, 72.9 (C-5^{a-g}, C-2^g), 71.6, 71.2, 71.1, 70.9, 70.8, 70.7 (C-2^{a-f}, C-4^{a-g}), 70.1 (NC(O)CH₂CH₂O), 68.1 (C(O)CH₂CH₂O), 66.7 (OCH₂CH₂CH₂N), 63.3 (SCH(CH₂)CHCH), 61.8, 61.6 (C-6^{a-i}, SCH(CH₂)CHCH), 56.6 (SCH(CH₂)CHNH), 40.9 (SCH(CH₂)CHCHCH₂), 40.2 (NC(O)CH₂CH₂O), 37.8 (OCH₂CH₂CH₂N), 37.4 (NC(O)CH₂CH₂O), 36.7 (C(O)CH₂CH₂CH₂CH₂), 29.5 (OCH₂CH₂CH₂N), 29.4, 29.1, 28.9 (C(O)CH₂CH₂CH₂CH₂, C(O)-CH₂CH₂CH₂CH₂, OCH₂CH₂CH₂N), 26.4 (C(O)CH₂CH₂CH₂CH₂); HRMS (ESI-TOF) m/z [M + H]⁺ calcd for C₆₉H₁₂₁N₄O₄₅S 1757.7024, found 1757.7012.

Conjugate of the Nonasaccharide with Biotin (21). A 0.062 M solution of biotin-activated ester **19** in DMF (48 μ L) and dry triethylamine (12 μ L) was added to a solution of **17** (3.5 mg, 0.0023 mmol) in DMF (100 μ L) + H₂O (100 μ L). After 12 h, the solvent was removed under reduced pressure, and the product was purified by gel-permeation chromatography (TSK HW-40(S), 0.1 M AcOH). The appropriate fractions were freeze-dried to give **21** (4 mg, 84%) as a fluffy solid. R_f 0.63 (n-butanol/ethanol/water/15% NH₄OH 0.5:1:0.8:0.4); ¹H NMR (600 MHz, D₂O) δ_H 5.40–5.36 (m, 8H, H-1^{b-i}), 4.93 (d, 1H, $J_{1^a,2^a}$ 3.8 Hz, H-1^a), 4.63 (dd, J 4.9 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.44 (dd, J 4.5 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.08–4.01 (m, 8H, H-5^{b-i}), 3.95–3.90 (m, 7H, H-3^{b-h}), 3.90–3.82 (m, 10H, H-3^a, H-6A^{a-i}), 3.83–3.74 (m, 12H, H-6B^{a-i}, H-3ⁱ, NC(O)CH₂CH₂O, OCH₂CH₂CH₂N^A), 3.73–3.63 (m, 39H, H-4^{a-h}, H-2^{a-h}, H-5^a, 5 OCH₂CH₂O, OCH₂CH₂N), 3.60–3.54 (m, 2H, H-2ⁱ, OCH₂CH₂CH₂N^B), 3.45 (t, $J_{4^i,3^i} = J_{4^i,2^i}$ 9.6 Hz, 1H, H-4ⁱ), 3.41 (t, J 5.4 Hz, 2H, OCH₂CH₂N), 3.38–3.31 (m, 3H, OCH₂CH₂CH₂N, SCH(CH₂)CHNH), 3.02 (dd, J 5.0 Hz, J 13.0 Hz, 1H, SCH(CH₂)CHCHCH₂^A), 2.80 (d, J 13.0 Hz, 1H, SCH(CH₂)CHCHCH₂^B), 2.54 (t, J 6.2 Hz, 2H, NC(O)CH₂CH₂O), 2.29 (t, J 7.3 Hz, 2H, C(O)CH₂CH₂CH₂CH₂), 1.89 (m, 2H, OCH₂CH₂CH₂N), 1.75 (m, 1H, C(O)CH₂CH₂CH₂CH₂^A), 1.71–1.58 (m, 3H, C(O)CH₂CH₂CH₂CH₂, C(O)CH₂CH₂CH₂CH₂^A), 1.47–1.41 (m, 2H, C(O)CH₂CH₂CH₂CH₂); ¹³C NMR (150.9 MHz, D₂O) δ_C 177.7, 174.7 (C(O)), 100.6, 100.44 (C-1^{b-i}), 99.5 (C-1^a), 81.4, 81.3 (C-3^{a-h}),

74.1 (C-3ⁱ), 73.1, 73.0, 72.9 (C-5^{a-i}, C-2ⁱ), 71.6, 71.2, 71.1, 70.9, 70.8, 70.7 (C-2^{a-h}, C-4^{a-i}), 70.1 (NC(O)CH₂CH₂O), 68.1 (C(O)CH₂CH₂O), 66.7 (OCH₂CH₂CH₂N), 63.3 (SCH(CH₂)CHCH), 61.8, 61.6 (C-6^{a-i}, SCH(CH₂)CHCH), 56.6 (SCH(CH₂)CHNH), 40.9 (SCH(CH₂)CHCHCH₂), 40.2 (NC(O)CH₂CH₂O), 37.8 (OCH₂CH₂CH₂N), 37.4 (NC(O)CH₂CH₂O), 36.7 (C(O)CH₂CH₂CH₂CH₂), 29.5 (OCH₂CH₂CH₂N), 29.1, 29.1, 28.9 (C(O)CH₂CH₂CH₂CH₂, C(O)-CH₂CH₂CH₂CH₂, OCH₂CH₂CH₂N), 26.4 (C(O)-CH₂CH₂CH₂CH₂); HRMS (ESI-TOF) m/z [M - 2H]²⁻ calcd for C₈₂H₁₄₂N₄O₅₅S 1046.4006, found 1046.3994.

Conjugate of the Undecasaccharide with Biotin (22). A 0.062 M solution of biotin-activated ester **19** in DMF (26 μ L) and dry triethylamine (6.4 μ L) was added to a solution of **18** (3 mg, 0.0016 mmol) in DMF (100 μ L) + H₂O (100 μ L). After 12 h, the solvent was removed under reduced pressure, and the product was purified by gel-permeation chromatography (TSK HW-40(S), 0.1 M AcOH). The appropriate fractions were freeze-dried to give **22** (1.9 mg, 49%) as a fluffy solid. R_f 0.60 (n-butanol/ethanol/water/15% NH₄OH 0.5:1:0.8:0.4); ¹H NMR (600 MHz, D₂O) δ_H 5.40–5.36 (m, 8H, H-1^{b-k}), 4.93 (d, 1H, $J_{1^a,2^a}$ 3.8 Hz, H-1^a), 4.62 (dd, J 4.9 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.44 (dd, J 4.5 Hz, J 8.0 Hz, 1H, SCH(CH₂)CHCH), 4.08–4.01 (m, 10H, H-5^{b-k}), 3.95–3.89 (m, 9H, H-3^{b-j}), 3.89–3.82 (m, 12H, H-3^a, H-6A^{a-k}), 3.83–3.74 (m, 14H, H-6B^{a-k}, H-3^k, NC(O)CH₂CH₂O, OCH₂CH₂CH₂N^A), 3.73–3.63 (m, 43H, H-4^{a-j}, H-2^{a-j}, H-5^a, 5 OCH₂CH₂O, OCH₂CH₂N), 3.60–3.54 (m, 2H, H-2^k, OCH₂CH₂CH₂N^B), 3.45 (t, $J_{4^k,3^k} = J_{4^k,2^k}$ 9.6 Hz, 1H, H-4^k), 3.41 (t, J 5.4 Hz, 2H, OCH₂CH₂N), 3.38–3.31 (m, 3H, OCH₂CH₂CH₂N, SCH(CH₂)CHNH), 3.02 (dd, J 5.0 Hz, J 13.0 Hz, 1H, SCH(CH₂)CHCHCH₂^A), 2.80 (d, J 13.0 Hz, 1H, SCH(CH₂)CHCHCH₂^B), 2.54 (t, J 6.2 Hz, 2H, NC(O)CH₂CH₂O), 2.29 (t, J 7.3 Hz, 2H, C(O)CH₂CH₂CH₂CH₂), 1.89 (m, 2H, OCH₂CH₂CH₂N), 1.75 (m, 1H, C(O)CH₂CH₂CH₂CH₂^A), 1.71–1.58 (m, 3H, C(O)CH₂CH₂CH₂CH₂, C(O)CH₂CH₂CH₂CH₂^A), 1.47–1.41 (m, 2H, C(O)CH₂CH₂CH₂CH₂); ¹³C NMR (150.9 MHz, D₂O) δ_C 177.7, 174.7 (C(O)), 100.6, 100.5, 100.44 (C-1^{b-k}), 99.5 (C-1^a), 81.4, 81.3 (C-3^{a-j}), 74.1 (C-3^k), 73.1, 73.0, 72.9 (C-5^{a-k}, C-2^k), 71.6, 71.2, 71.1, 70.9, 70.7 (C-2^{a-j}, C-4^{a-k}), 70.1 (NC(O)CH₂CH₂O), 68.1 (C(O)CH₂CH₂O), 66.7 (OCH₂CH₂CH₂N), 63.3 (SCH(CH₂)CHCH), 61.8, 61.5 (C-6^{a-k}, SCH(CH₂)CHCH), 56.6 (SCH(CH₂)CHNH), 40.9 (SCH(CH₂)CHCHCH₂), 40.2 (NC(O)CH₂CH₂O), 37.8 (OCH₂CH₂CH₂N), 37.4 (NC(O)CH₂CH₂O), 36.7 (C(O)CH₂CH₂CH₂CH₂), 29.5 (OCH₂CH₂CH₂N), 29.1, 29.1, 28.9 (C(O)CH₂CH₂CH₂CH₂, C(O)-CH₂CH₂CH₂CH₂, OCH₂CH₂CH₂N), 26.4 (C(O)-CH₂CH₂CH₂CH₂); HRMS (ESI-TOF) m/z [M + 2H]²⁺ calcd for C₉₄H₁₆₄N₄O₆₅S 1210.9697, Found 1210.9697.

Biological Assays.

Coating of Streptavidin Microtiter Plates with Biotinylated α -(1 $\rightarrow$ 3)-Glucan

Oligosaccharides. Biotinylated α -(1 $\rightarrow$ 3)-glucan oligosaccharides **20–24** were loaded on streptavidin-coated 96-well microtiter plates (R&D Systems or Pierce/ThermoFisher) as described.²⁵ The streptavidin-coated plates were washed once with PBS supplemented with 1% bovine serum albumin (PBS-BSA). The oligosaccharides were diluted at 100 ng/mL in PBS-BSA, and 100 μ L of solution was added to each well. After incubation for 1 h at room temperature, the plates were extensively washed with PBS-BSA.

Use of Streptavidin Plates Coated with Oligosaccharides for the Characterization and Detection of Specific Anti- α -(1 $\rightarrow$ 3)-Glucan Antibodies and Epitope Identification. The oligosaccharide-coated plates were incubated with the monoclonal antibodies (diluted 1:250 in PBS-BSA) or human sera (diluted 1:500 in PBS-BSA in triplicates) at room temperature for 1 h.

A secondary antibody (goat antimouse IgG (whole molecule) conjugated with HRP or antihuman IgG-Fc antibody conjugated with HRP (Sigma-Aldrich) (both 1:1000 in PBS-BSA) was added to each well, and the plates were incubated for 1 h at room temperature. After washing, the recognition was quantified by O-phenylenediamine (OPD) with OD readings at 492 nm. A total of 122 serum samples from 49 aspergillosis patients and 21 healthy individuals were collected with written consent from Angers Hospital (Angers, France). The aspergillosis patients included those with allergic bronchopulmonary aspergillosis (ABPA) (26 patients, 47 serum samples) and chronic pulmonary aspergillosis (CPA) (23 patients, 53 serum samples).

The immunogenicity of the α -(1 $\rightarrow$ 3)-glucan biotinylated oligosaccharides was compared to that of native α -(1 $\rightarrow$ 3)-glucan. Cell wall α -(1 $\rightarrow$ 3)-glucan was extracted from *A. fumigatus* mycelium as described previously.²⁶ Microtiter plates (96-wells, Greiner) were coated with 25 μ g/mL native cell wall α -(1 $\rightarrow$ 3)-glucan in carbonate buffer (50 mM, pH 9.6) overnight. The wells were extensively washed with PBS supplemented with 0.05% Tween 20 and blocked for 1 h with PBS-BSA as described above for the streptavidin plates.

Immunolabeling of Germ Tubes of A. fumigatus by Anti- α -(1 $\rightarrow$ 3)-Glucan Monoclonal Antibodies. The *A. fumigatus* strain CEA17_ *akuB*^{KU80} (Ku80) and a triple α -(1 $\rightarrow$ 3)-glucan synthase mutant (*ags1/ags2/ags3*) with a cell wall devoid of α -(1 $\rightarrow$ 3)glucan were used.⁴ Conidia (5×10^5 conidia/mL) was inoculated on eight-well glass-bottom α -slides (Ibidi, Martinsried, Germany) in 250 μ L of Brian medium²⁷ at 30 °C for 18 h. Germinated conidia were permeabilized as described previously.²⁸ Briefly, germinated conidia were fixed with 2.5% p-formaldehyde overnight at 4 °C and washed with phosphate-buffered saline pH 7.0 (PBS) containing 0.1 M NH₄Cl and then with PBS. Fixed germinated conidia were permeabilized by successive incubation in Glucanex (Novozymes) for cell wall removal in the extraction solution containing Nonidet P-40 detergent and then in methanol. Immunolabeling was performed as previously described.⁴ Permeabilized germinated conidia were incubated with the anti- α -(1 $\rightarrow$ 3)-glucan monoclonal antibodies A-16 (IgA) or J558 (IgM) (8 μ g/mL)¹⁷ followed by an antimouse IgG conjugated to Alexa488 (antimouse IgG-A488, 1:500 diluted; Sigma). Samples were observed under a fluorescence microscope. MD4-4 (antihen egg lysosome IgM) was used as the negative control.

Use of Streptavidin Plates Coated with Oligosaccharides To Monitor the Induction of Cytokines. PBMCs were isolated from whole-blood samples obtained from Hôpital Saint-Louis (Paris, France), through the Etablissement Francais du Sang (Paris, France), from four healthy donors with written consent. The use of this material was approved by the ethics committees of Institut Pasteur and the Etablissement Francais du Sang (convention 12/EFS/023)., PBMCs were isolated by a Ficoll-Hypaque density gradient. First, the whole-blood samples were diluted in PBS (ratio 1:1). The diluted blood was then layered on top of

15 mL of Ficoll-Hypaque in 50 mL sterile polystyrene conical bottom tubes and centrifuged at 1800 rpm for 20 min at 22 °C. The buffy coat interface was collected and washed once with sterile PBS by centrifugation at 1500 rpm for 10 min at 22 °C. To remove the platelets, the PBMCs were washed again with sterile PBS by centrifugation at 1200 rpm for 10 min at 22 °C. Finally, the PBMCs were resuspended in 20 mL of RPMI-1690 medium supplemented with 10% normal human serum (ZenBio Inc., USA). The number of viable PBMCs per milliliter was counted by a Luna-FLTM dual fluorescence cell counter with acridine orange/propidium iodide cell viability kit (Logos Biosystems, South Korea). PBMCs (2.5×10^5 cells/well in RPMI supplemented with 10% normal human serum) were added to the wells of the streptavidin-coated plates that had been precoated with the oligosaccharides. The supernatant was collected after 24 h of incubation at 37 °C in a humid chamber with a 5% CO₂/95% air atmosphere. The supernatant was stored at -20 °C until further quantification of the TNF- α , IL-6, IL-1 β and IL-1Ra levels by DuoSet ELISA kits following the manufacturer's instructions (R&D Systems). The induction of cytokines by free oligosaccharides was examined in nonstreptavidin plates. In addition, the induction of cytokines by high-molecular-weight water-insoluble α -(1 $\rightarrow$ 3)-glucan (10 μ g/mL) in suspension or loaded on nonstreptavidin plates was examined with the same procedures.

Statistical Analysis. Data analyses were performed by one-way ANOVA nonparametric tests (Kruskal–Wallis or Friedman tests) with Dunn's multiple comparison test in GraphPad software Prism. A *p*-value <0.05 was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Detection of anti- α -(1 $\rightarrow$ 3)-glucan antibodies in the sera of patients with *Aspergillus* infections by enzyme-linked immunosorbent assay (ELISA) with oligosaccharide ligands **20–24** and α -(1 $\rightarrow$ 3)-glucan. The mean OD values of triplicate evaluations of each serum in the two patient groups with allergic bronchopulmonary aspergillosis (ABPA) or chronic pulmonary aspergillosis (CPA) were plotted in the graphs. The bars are the median of the mean OD values of the sera. The median OD values of both the ABPA and CPA serum groups obtained with the biotinylated oligosaccharides and the native α -(1 $\rightarrow$ 3)-glucan were all significantly higher than those of the control serum group (without *Aspergillus* infection) when compared by Kruskal–Wallis test and Dunn’s multiple comparison test (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$).

Figure 2.

Identification of the oligo- α -(1 $\rightarrow$ 3)-glucan epitope recognized by monoclonal anti- α -(1 $\rightarrow$ 3)-glucan antibodies. Biotinylated α -(1 $\rightarrow$ 3)-glucan oligosaccharides were bound to streptavidin-coated plates, and natural α -(1 $\rightarrow$ 3)-glucan was loaded onto usual (streptavidin-uncoated) plates. The optical density (OD) value indicated the level of binding between the two monoclonal anti- α -(1 $\rightarrow$ 3)-glucan antibodies (A16 and J558) and the oligo- α -(1 $\rightarrow$ 3)-glucan. An antihen egg lysozyme IgM antibody (MD4-4) was used as a control. All the oligo- α -(1 $\rightarrow$ 3)-glucans were recognized by both monoclonal anti- α -(1 $\rightarrow$ 3)-glucan antibodies (A16 and J558), whereas the natural α -(1 $\rightarrow$ 3)-glucan was not recognized.

Figure 3.

Levels of cytokine formation induced by the different biotinylated α -(1 $\rightarrow$ 3)-glucan oligosaccharides. PBMCs of four donors were coincubated with biotinylated α -(1 $\rightarrow$ 3)-glucan oligosaccharides immobilized on streptavidin microtiter plates or with free natural high-molecular-weight water-insoluble α -(1 $\rightarrow$ 3)-glucan (10 μ g/mL) in suspension on nonstreptavidin plates. The coincubation was performed in RPMI supplemented with 10% normal human serum at 37 $^{\circ}$ C for 24 h. Thereafter, the supernatant was collected for quantification of the cytokines (A) TNF- α , (B) IL-6, (C) IL-1 β and (D) IL-1Ra by ELISA. The dots represent the mean cytokine concentration of triplicate evaluations of samples from each donor, and the lines represent the median of the four mean values. The cytokine concentrations induced by the oligosaccharides and the native α -(1 $\rightarrow$ 3)-glucan were compared to that of the control by nonparametric one-way ANOVA (Kruskal–Wallis test) with Dunn's multiple comparison (**** p < 0.0001, *** p < 0.001, ** p < 0.01, * p < 0.05, ns = not significant). The length of the oligosaccharides is directly proportional to the level of cytokine induction. Undecasaccharide **22** and nonasaccharide **21** significantly induced the production of all tested cytokines, while the shorter oligosaccharides, **20**, **23**, and **24**, did not (A–D). Although some cytokine induction is observed with the natural α -(1 $\rightarrow$ 3)-glucan, the induction was only statistically significant for IL-1Ra (D).

Scheme 1.

Assembly of Hepta-, Nona- and Undecaglycosides 8, 10 and 11 Related to Fragments of *A. fumigatus* α -(1 $\rightarrow$ 3)-Glucan^a

^aReagents and conditions: (a) N₂H₄·H₂O, AcOH, (b) MeOTf, CH₂Cl₂, -35 °C $\rightarrow$ 0 °C, (c) CAN, H₂O, benzene, CH₃CN, (d) CF₃C(NPh)Cl, K₂CO₃, acetone.

Scheme 2.

Deprotection of Hepta-, Nona- and Undecasaccharides 9, 10, and 11 and Preparation of Biotinylated Conjugates 20–22^a

^aReagents and conditions: (a) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , MeOH, EtOAc, (b) NaOH, MeOH, (c) $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, AcOH, (d) NaOH, H_2O , CH_2Cl_2 , MeOH, (e) Na, NH_3 , THF, -78°C , (f) **19**, Et_3N , DMF.