Bioconjugate Chemistry

Article

pubs.acs.org/bc

Synthesis and Immunological Studies of Linear Oligosaccharides of β-Glucan As Antigens for Antifungal Vaccine Development

Guochao Liao,[†,∥] Zhifang Zhou,[†,∥] Srinivas Burgula,[†] Jun Liao,[†,‡] Cheng Yuan,[§] Qiuye Wu,[‡] and Zhongwu Guo*[,†]

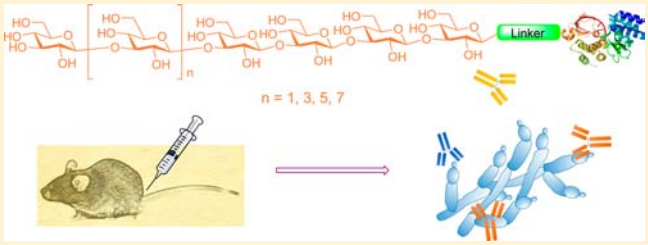
[†]Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, United States

[‡]School of Pharmacy, Second Military Medical University, 325 Guohe Road, Shanghai 200433, China

[§]Department of Pharmacy, Shanghai East Hospital, Tongji University, 150 Jimo Road, Shanghai 200120, China

Supporting Information

ABSTRACT: Antifungal vaccines have recently engendered considerable excitement for counteracting the resurgence of fungal infections. In this context, β-glucan, which is abundantly expressed on all fungal cell surfaces, functionally necessary for fungi, and immunologically active, is an attractive target antigen. Aiming at the development of effective antifungal vaccines based on β-glucan, a series of its oligosaccharide derivatives was designed, synthesized, and coupled with a carrier protein, keyhole limpet hemocyanin (KLH), to form new semisynthetic glycoconjugate vaccines. In this article, a convergent and effective synthetic strategy using preactivation-based iterative glycosylation was developed for the designed oligosaccharides. The strategy can be widely useful for rapid construction of large oligo-β-glucans with shorter oligosaccharides as building blocks. The KLH conjugates of the synthesized β-glucan hexa-, octa-, deca-, and dodecasaccharides were demonstrated to elicit high titers of antigen-specific total and IgG antibodies in mice, suggesting the induction of functional T cell-mediated immunity. Moreover, it was revealed that octa-, deca-, and dodeca-β-glucans were much more immunogenic than the hexamer and that the octamer was the best among these. The results suggested that the optimal oligosaccharide sequence of β-glucan required for exceptional immunogenicity was a hepta- or octamer and that longer glucans are not necessarily better antigens, a finding that may be of general importance. Most importantly, the octa-β-glucan−KLH conjugate provoked protective immunity against *Candida albicans* infection in a systemic challenge model in mice, suggesting the great potential of this glycoconjugate as a clinically useful immunoprophylactic antifungal vaccine.



INTRODUCTION

Fungal infection poses a great threat to human health, and its cases grow rapidly year by year[1] owing to the limitations of current antifungal drugs and, especially, the emergence of drug-resistant strains. As a result, deep-seated infections in nosocomial settings have a high mortality even after treatment with antifungal drugs.[2,3] Moreover, many commensal and opportunistic fungi, previously thought to be nonpathogenic, have emerged as pathogens in immunocompromised patients.[4,5] To meet the urgent medical need for antifungal therapies, development of prophylactic and/or therapeutic antifungal vaccines is increasingly considered to be one of the most attractive and appropriate strategies.[6]

Beta-(1,3)-glucan (β-glucan) with sporadic branches linked to the 6-*O*-position is an essential cell wall component of various fungi.[7] This biopolymer is exposed on the surface of fungal cells and is functionally necessary; thus, it is an excellent target antigen for the development of broadly useful antifungal vaccines. It has been demonstrated that β-glucan can provoke immunogenic protection against *Candida albicans* in mice.[8] Therefore, a series of vaccines based on natural β-glucans, such as their conjugates with diphtheria toxin CRM_{197}, has been explored and shown to elicit protection against *Candida* in a mouse model.[9−11] Recent studies suggested that linear β-glucan and its short oligosaccharides could elicit immune responses and protection against *C. albicans*.[12−14] Furthermore, it has been demonstrated that the CRM_{197} conjugate of a synthetic β-glucan hexasaccharide elicited stronger glucan-specific antibody responses than the conjugate of natural β-glucan. As an antifungal vaccine, the hexasaccharide conjugate was proposed to be more promising than other synthetic or natural β-glucan conjugates.[12−14]

Developing conjugate vaccines using synthetic oligosaccharide antigens is a relatively new concept. One of the successful stories of this approach was the development of a semisynthetic anti-*Haemophilus influenzae* type b (Hib) vaccine.[15] This type of vaccine has some advantages. For example, its synthetic antigens have defined chemical structures, which would facilitate detailed immunological and structure−activity relationship studies to help gain more insight into the function of

Received: December 7, 2014
Revised: January 20, 2015
Published: February 11, 2015

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

the vaccine and to optimize vaccine design. The reaction sites and/or linkage positions of the carbohydrate antigens are well-defined and predictable, which would improve vaccine quality control. Therefore, as promising candidate antigens for vaccine design, oligomeric β-glucans have received considerable attention. For example, several groups have recently synthesized linear tetra,[16] penta,[17] hexa,[14,18,19] dodeca,[20] hexadeca,[21] and branched heptadeca oligosaccharides[21] of β-glucans by various methods. However, most conjugate vaccines studied so far are made of heterogeneous natural β-glucans or oligosaccharides derived from natural β-glucans, and there are very few reports about conjugate vaccines derived from synthetic β-glucan derivatives.[12−14]

In an effort to systematically explore the structure−immunogenicity relationships of linear β-glucans and to optimize the design of the antigen for creating more efficacious antifungal vaccines, we have (1) developed a highly convergent and effective method for the synthesis of oligosaccharides of β-glucan with varied chain lengths, (2) coupled them with keyhole limpet hemocyanin (KLH), and (3) evaluated the immunological properties of resulting glycoconjugates and their capability to elicit protective immune responses against *C. albicans* in mice.

RESULTS AND DISCUSSION

On the basis of reports that a hexasaccharide of β-glucan was highly immunogenic[12−14] and that at least an octa- or nonasaccharide may be required to generate special 3D structures,[22] we planned to prepare and compare hexa-, octa-, deca- and dodecasaccharides of β-glucan (Figure 1). These

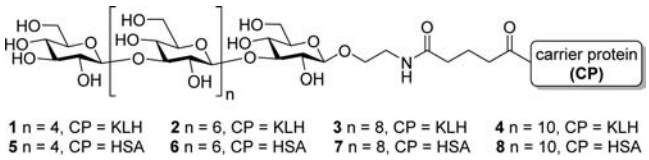


Figure 1. Structure of designed β-glucan oligosaccharides and their protein conjugates **1**−**8**.

oligosaccharides were coupled with KLH to form vaccines **1**−**4**. KLH is one of most commonly used and effective protein carriers for glycoconjugate vaccine development.[23] In the meantime, the oligosaccharides were also coupled with human serum albumin (HSA) to provide conjugates **5**−**8** that were used as capture reagents for detecting β-glucan-specific antibodies by enzyme-linked immunosorbent assay (ELISA).

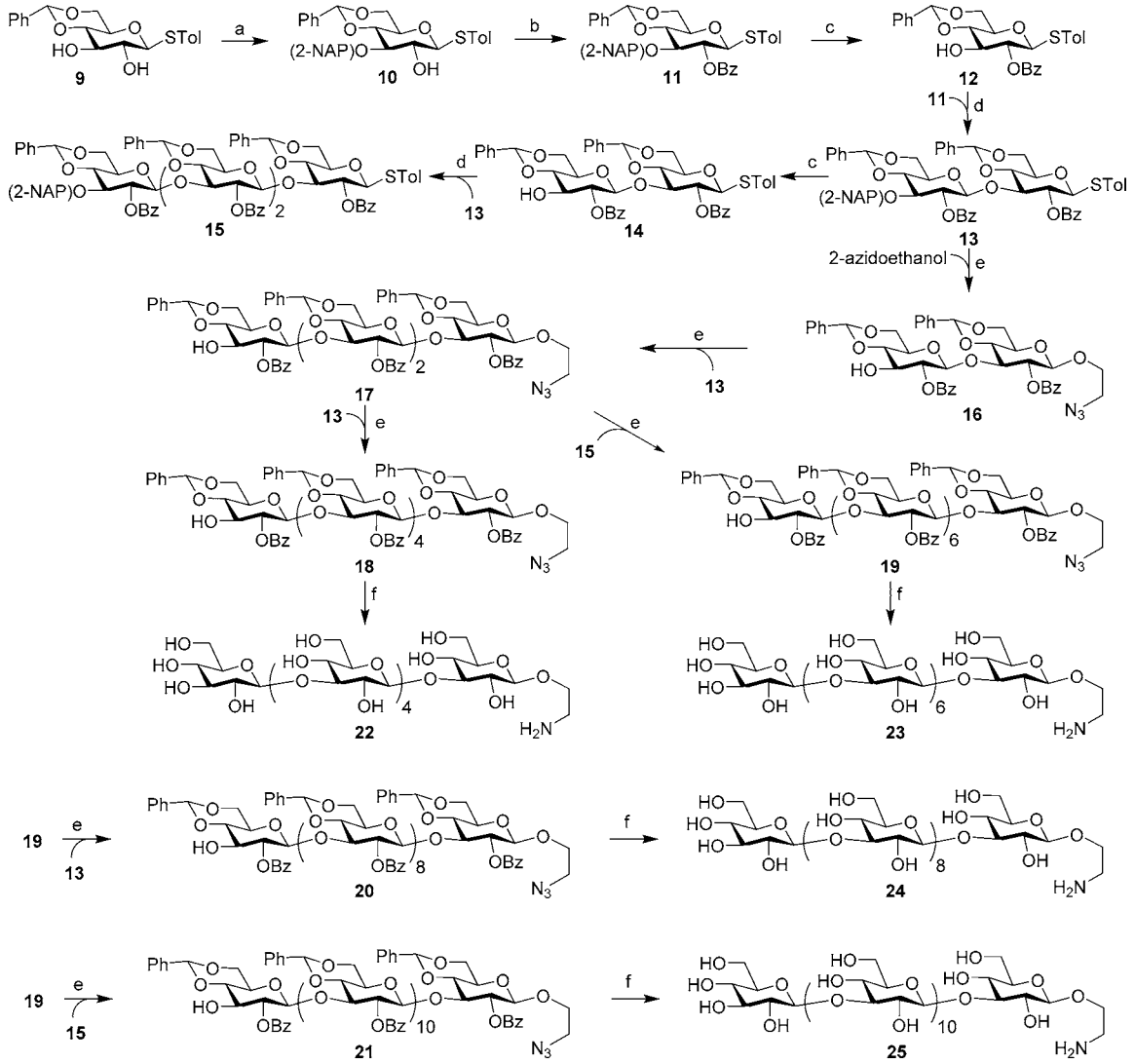
Oligosaccharide Synthesis. As depicted in Scheme 1, the designed β-glucan oligosaccharides were achieved through preactivation-based iterative glycosylation with *p*-toluenethioglycosides as glycosyl donors and disaccharide **13** as a key building block. The synthesis was commenced with the preparation of **9** from D-glucose according to a literature procedure in four steps and in 40% overall yield.[24,25] Treatment of **9** with dibutyltin oxide to furnish the stannylene acetal-directed regioselective protection of the 3-*O*-position with a 2-naphthylmethyl (NAP) group[26,27] was followed by 2-*O*-benzoylation of the resultant **10** to afford thioglycoside donor **11**. Here, the NAP group was employed as a temporary protecting group instead of the common *para*-methoxybenzyl (PMB) group because the former is more stable under the acidic conditions involved in glycosylation reactions, although both groups can be readily removed with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[22,23] Removal of the 3-*O*-NAP group in **11** with DDQ was straightforward, giving **12** in excellent yield (92%).[26,27] Thereafter, **11** was coupled with **12** via preactivation glycosylation[28,29] to obtain **13**. Specifically, glycosyl donor **11** was first activated with *p*-toluenesulfenyl triflate (*p*-TolSOTf) that was generated *in situ* from the reaction between *p*-toluenesulfenyl chloride (*p*-TolSCl) and silver triflate (AgOTf) at −78 °C. Then, glycosyl acceptor **12** was added to furnish glycosylation, resulting in desired β-disaccharide **13** ($J_{1,2}$ = 7.5 Hz, 90% yield) in a stereospecific manner, due to neighboring group participation. Compound **13** was used as one of the common glycosyl donors for subsequent carbohydrate chain elongation. Removal of the NAP group in **13** with DDQ provided **14**. A convergent [2 + 2] glycosylation reaction between **13** and **14** by the same preactivation protocol yielded tetrasaccharide **15** (86%) as a glycosyl donor for more complex oligosaccharide assembly. Preactivated glycosylation of 2-azidoethanol with **13**, followed by removal of the NAP group with DDQ, afforded **16** (91%), which carried an azido group at the nonreducing end. The azido group would be reduced to form a primary amine later on to enable a selective reaction with the linker and then coupling with carrier proteins. Moreover, since the preactivation-based glycosylation reaction was clean and high yielding and the donor and acceptor were almost completely consumed, this allowed us to move on to the next step after glycosylation, i.e., removal of the NAP group, without purification of the reaction intermediate. Similarly, preactivation-based glycosylation of **16** with **13** and then removal of the NAP group produced tetrasaccharide **17**. On the basis of **17**, the sugar chain was further elongated successfully via preactivation-based glycosylation to achieve all of the designed β-glucan oligosaccharides. Coupling of **17** with disaccharide **13** and tetrasaccharide **15**, followed by selective removal of the NAP group, afforded hexasaccharide **18** and octasaccharide **19**, respectively. Subsequently, **19** was coupled with **13** and **15**, which was followed by NAP group removal to produce decasaccharide **20** and dodecasaccharide **21**. Notably, the glycosylation yields were not significantly affected by the increased size of the involved building blocks. All of the synthetic intermediates and final products were fully characterized, proving that the glycosylation reactions were β-specific.

Attempts to globally deprotect **18**−**21** via saponification followed by hydrogenation or via hydrogenation followed by saponification in one pot were unsuccessful, mainly due to the poor mutual solubility of **18**−**21** and the partially deprotected products in various solvent combinations. Eventually, they were fully deprotected in a stepwise manner using the proper solvent or solvent combination for each transformation, following the order of Zn-mediated reduction of the azido group in dichloromethane (DCM), acidic cleavage of all benzylidene groups in acetic acid and water (5:1), and finally sodium hydroxide-promoted removal of all benzoate groups in *t*-butyl alcohol and water (4:1). The final products were purified with a Sephadex-G25 size-exclusion column with distilled water as the eluent to afford **22**−**25** as white fluffy solids upon lyophilization. The final products were fully characterized by both NMR and MS.

Conjugation of Oligosaccharides 22−25 with Carrier Proteins. Free oligosaccharides **22**−**25** were conjugated with carrier proteins KLH and HSA through the bifunctional glutaryl group, as this conjugation can be easily and effectively realized via activated glutaryl ester and, not like other complex linkers, this simple linker would not affect the immunological activity of

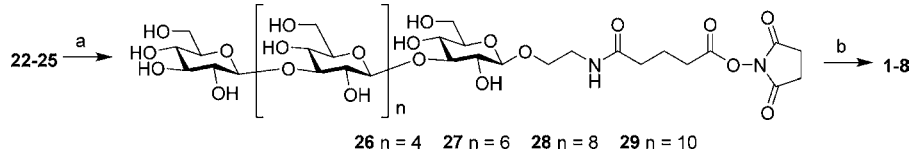
DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

Scheme 1. Synthesis of β-Glucan Oligosaccharides 22−25[a]



[a]Reagents and conditions: (a) Bu_2SnO, toluene, reflux, 6 h; then 2-naphthylmethyl bromide, CsF, DMF, 70 °C, 12 h, 72%; (b) BzCl, Et_3N, CH_2Cl_2, rt, 12 h, 96%; (c) DDQ, CH_2Cl_2/H_2O (18:1), rt, 8 h, 92% for **12**, 95% for **14**; (d) AgOTf, TTBP, *p*-TolSCl, CH_2Cl_2, −78 °C to rt, 4 h, 90% for **13**, 86% for **15**; (e) AgOTf, TTBP, *p*-TolSCl, CH_2Cl_2, −78 °C, rt, 4 h; then DDQ, CH_2Cl_2/H_2O (18:1), rt, 8 h, 91% for **16**, 90% for **17**, 87% for **18**, 81% for **19**, 80% for **20**, 85% for **21**; (f) Zn, AcOH, CH_2Cl_2, 24 h, rt; then $AcOH/H_2O$ (5:1), 60 °C, 24 h; finally NaOH, *t*-$BuOH/H_2O$, 40 °C, 24 h, 80% for **22**, 88% for **23**, 85% for **24**, 88% for **25**.

Scheme 2. Preparation of Oligo-β-glucan−Protein Conjugates 1−8[a]



26 n = 4 **27** n = 6 **28** n = 8 **29** n = 10

[a]Reagents and conditions: (a) DSG, DMF, and PBS buffer (4:1), rt, 4 h; (b) KLH or HSA, PBS buffer, rt, 2.5 days.

conjugates.[30,31] A two-step procedure was used to furnish the conjugation (Scheme 2). First, reaction between the free amino group in **22−25** and a large excess of active ester disuccinimidal glutarate (DSG) gave the corresponding monoactivated esters **26−29** in quantitative yields. Then, **26−29** were coupled with KLH or HSA in 0.1 M phosphate-buffered saline (PBS) to afford desired glycoprotein conjugates **1−8**, which were purified with a Biogel A0.5 column to remove remaining free sugars. The conjugate-containing fractions were dialyzed against distilled water and lyophilized to give **1−8**. Finally, the glucose content of each conjugate was analyzed by the phenol−sulfuric acid method following a reported protocol.[32] The glucose content of the KLH and HSA conjugates was 7.5−9.1% and 10.5−25.8%, respectively (Table 1), showing that the coupling reactions were efficient and the antigen loading levels were in the desired range for glycoconjugate vaccines.[33] The sugar loading of HSA conjugates was also confirmed by MALDI-TOF mass spectrometry.

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

Table 1. Carbohydrate Loading of Glycoconjugates 1−8

	KLH conjugates				HSA conjugates			
sample	1	2	3	4	5	6	7	8
loading (%)	8.3	7.8	7.5	9.1	10.5	11.0	14.3	25.8

Immunological Studies of Glycoconjugate Vaccines 1−4. The immunological properties of KLH conjugates **1**−**4** were investigated in female C57BL/6J mice. For this purpose, each conjugate was thoroughly mixed with Titermax Gold adjuvant, and the resulting emulsion was then injected intramuscularly (i.m.) into mice. Following the initial immunization, mice were boosted 4 times on days 14, 21, 28, and 38 by subcutaneous (s.c.) injection of the same vaccine emulsion. Blood samples of each mouse were collected through the leg veins prior to the initial immunization on day 0 and after immunizations on days 27, 38, and 48. Antisera were obtained from clotted blood samples and were stored at −80 °C before use. ELISA using the corresponding HSA conjugates as capture reagents for plate coating was employed to determine antibody titers, which reflected the elicited immune responses. Antibody titers were defined as the dilution number yielding an OD value of 0.2, and the results are shown in Figure 2.

Evidently, all of the KLH conjugates **1**−**4** elicited high titers of antigen-specific total (kappa) antibodies, indicating strong immune responses. More importantly, high titers of IgG1 antibodies were observed for all glycoconjugates, suggesting memorable T cell-dependent immunities.[34,35] IgG1 antibody is usually considered to be the protective antibody isotype;[36,37] thus, these conjugates were believed to elicit protective immune responses and have great potential for being developed into clinically functional vaccines against fungal infections.

The above immunological results revealed that, overall, conjugates **2**−**4** induced significantly higher titers of both total (anti-kappa) and IgG1 antibodies than **1** ($P \ll 0.01$, Figure 3), indicating that **2**−**4** were much more immunogenic and provoked much stronger immune responses in mice than **1**. Further analysis of the immune responses showed that the IgG1 antibody titer induced by **2** was significantly higher than that induced by **3** and **4** ($P < 0.01$, Figure 3B) as well. Although the total antibody titer for **2** was also slightly higher than that for **3** and **4** (Figure 3A), this difference was less significant ($P > 0.05$ and <0.01, respectively). There are several factors that may affect the immune response to a glycoconjugate, such as carbohydrate loading,[38,39] conjugation method, immunization protocol, and carbohydrate antigen structure. The carbohydrate loadings of **1**−**4** were very similar, and their conjugation method and immunization protocol were identical. Therefore, the different immunological properties for these glycoconjugates were because of their different carbohydrate structures, and, among the oligosaccharides investigated here, octa-β-glucan seemed to be the most immunogenic and the most promising antigen for vaccine development.

Protection against Fungal Infection in Mice. To ultimately prove the efficacy of the new glycoconjugate vaccine to protect against fungal infections, conjugate **2**, which elicited the strongest immune responses in the above studies, was evaluated in a fungal challenge experiment in mice. The fungus used was *C. albicans* (strain SC5314), one of the most common and important pathogenic fungi in clinic.[40] In this experiment, each group of 11 mice was immunized with **2** or PBS (the control group) 4 times on days 1, 14, 21, and 28 according to the above-mentioned protocols. On day 38, a predetermined lethal dose of *C. albicans* (7.5×10^5 cells/mouse in 200 μL

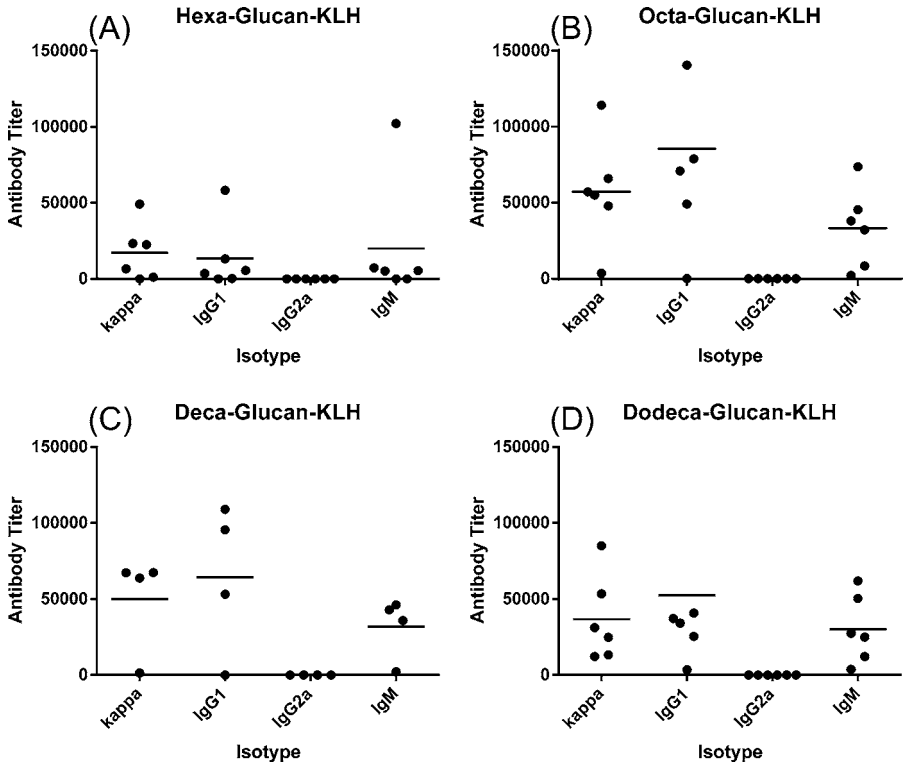


Figure 2. ELISA results of the day 48 antisera obtained with conjugates **1** (A), **2** (B), **3** (C), and **4** (D) combined with Titermax Gold adjuvant, respectively. The titers of corresponding antigen-specific antibodies are displayed. Each dot represents the antibody titer of an individual mouse, and the black bar shows the average titer.

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

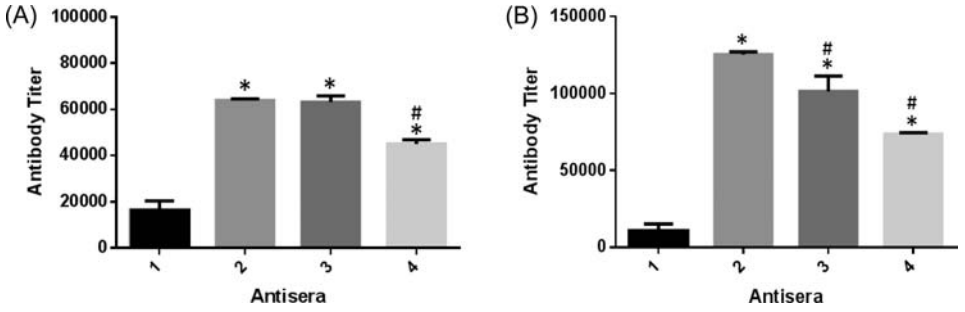


Figure 3. Comparison of the average antibody titers of corresponding antigen-specific (A) total (anti-kappa) antibody and (B) IgG1 antibody in the day 48 pooled antisera of mice immunized with conjugates **1**−**4**. Each error bar is the standard deviation of three parallel experiments. *, $P \ll 0.01$ compared to **1**; #, $P < 0.05$ compared to **2**.

PBS) was given by i.v. injection to each mouse. The responses of these mice were observed under normal feeding and care conditions. As shown in Figure 4, mice in the control group

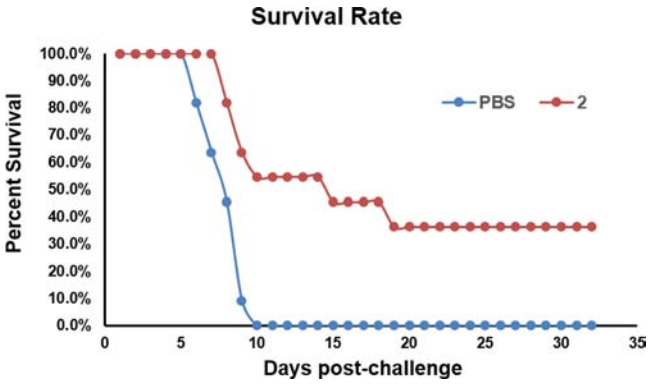


Figure 4. Survival time of mice immunized with **2** compared with mice immunized with PBS after i.v. injection of *C. albicans* (7.5×10^5 cells per mouse and 11 mice per group).

started to die of infection on day 6 after the fungal injection, and all died within 4 days (on day 10). In comparison, mice in the **2**-immunized group did not have a fatal incident until day 8, and on day 14, the animal survival rate was about 55%. At the end of this experiment (on day 32), there were still 4 mice (about 34%) in the immunized group unaffected, suggesting complete protection of these mice from *C. albicans* infection. These results proved that glycoconjugate **2** could elicit protective immunity in mice against lethal systemic challenge with *C. albicans*.

CONCLUSIONS

In summary, a series of β-glucan oligosaccharides was synthesized and coupled with KLH to generate glycoconjugates that contained structurally well-defined carbohydrate antigens. These glycoconjugates were shown to elicit robust T cell-dependent and protective immune responses in mice, which helped to identify some promising antifungal vaccines. This work is distinguished from previous studies in the area in several aspects. First, a highly convergent, effective, and potentially broadly applicable strategy was developed for the synthesis of structurally well-defined β-glucans. Large oligosaccharides could be rapidly assembled from short oligosaccharide segments by the preactivation-based glycosylation protocol that had significantly reduced the number of steps for anomeric manipulation. It was further observed that the size of the oligosaccharide segments used for the synthesis had little influence on the glycosylation efficiency. Furthermore, with the help of neighboring group participation, all of the glycosylations were highly stereoselective to create the desired β-anomer. Therefore, this synthetic strategy can be widely applicable to larger and more complex β-glucan derivatives via $[n + n]$ or $[n + (n + 1)]$ glycosylations.

Second, the synthesized oligosaccharides had a reactive amino group at their reducing ends, enabling their effective coupling with carrier proteins, such as KLH, through a bifunctional linker. The resultant conjugates contained structurally defined carbohydrate antigens to facilitate detailed and in-depth investigation of glycoconjugate vaccines, such as structure−activity relationship analysis. Although a number of β-glucan oligosaccharides have been synthesized previously, only a few have been conjugated with a carrier protein and investigated as vaccines. On the other hand, conjugate vaccines currently employed for biological studies are typically made of heterogeneous natural β-glucans or oligosaccharides derived from natural β-glucans.

Third, immunological studies of glycoconjugate vaccines **1**−**4** revealed that while all of them could elicit robust T cell-dependent immune responses, octa-, deca-, and dodeca-β-glucans were much more immunogenic than hexa-β-glucan, which was different from the literature results.[14] Close structure−activity relationship analysis further revealed that the immunogenicity decreased in the order of octa-, deca-, and dodeca-β-glucans. These results suggest that at least an octamer is necessary for oligo-β-glucans to be optimal antigens for eliciting functional immune responses. However, this does not necessarily mean that the longer the better for an oligosaccharide antigen. As a result, an octa- or nona-β-glucan was identified as the most promising antigen for designing and developing β-glucan-based antifungal vaccines, which is of general significance.

Finally and most importantly, we have demonstrated in a mouse model that the conjugate of KLH and octa-β-glucan, namely, **2**, could elicit protective immune responses against the deadly pathogen, *C. albicans*. This result is highly relevant to clinic application. Therefore, this work has paved the foundation for developing an effective and clinically useful antifungal vaccine.

EXPERIMENTAL SECTION

General Experimental Methods. Chemicals and materials were obtained from commercial sources and were used as received without further purification unless otherwise noted. MS 4 Å were flame-dried under high vacuum and used immediately after cooling under a N_2 atmosphere. Analytical

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

TLC was carried out on silica gel 60 Å F_{254} plates with detection by a UV detector and/or by charring with 15% (v/v) H_2SO_4 in EtOH. NMR spectra were recorded on a 400, 500, or 600 MHz machine, with chemical shifts reported in ppm (δ) downfield from tetramethylsilane (TMS), which was used as an internal reference.

*p-Tolyl 4,6-O-Benzylidene-3-O-(naphthalene-2-ylmethyl)-1-thio-β-D-glucopyranoside (**10**).* A mixture of diol **9** (9.0 g, 24.03 mmol) and Bu_2SnO (7.18 g, 28.84 mmol) in anhydrous toluene (400 mL) was refluxed in a flask equipped with a Dean−Stark device for 6 h. After the mixture was cooled to room temperature, the residual solvent was removed under vacuum. CsF (7.99 g, 52.87 mmol), 2-bromomethylnaphthalene (10.10 g, 45.66 mmol), and DMF (60 mL) were added, and the resulting solution was stirred at 70 °C for 12 h, when TLC showed completion of the reaction. After DMF was removed under vacuum, the residue was dissolved in CH_2Cl_2 and washed with 1 M NaF aqueous solution. The organic phase was dried over Na_2SO_4 and purified by flash column chromatography (toluene/EtOAc 10:1) to give **10** (8.9 g, 72%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85−7.70 (m, 4H), 7.51−7.40 (m, 10H), 7.15 (d, J = 7.83 Hz, 2H), 5.60 (s, 1H Ph-CH), 5.12 (d, J = 11.74 Hz, 1H), 4.99 (d, J = 11.74 Hz, 1H), 4.57 (d, J = 9.29 Hz, 1H, H-1), 4.40 (dd, J = 10.27, 4.89 Hz, 1H), 3.81 (t, J = 10.27 Hz, 1H), 3.75 (t, J = 9.29 Hz, 1H), 3.68 (t, J = 9.29 Hz, 1H), 3.56−3.48 (m, 2H), 2.61 (br s, 1H), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 138.8, 137.2, 135.6, 133.8, 135.3, 133.0, 129.9, 129.1, 128.3, 128.3, 127.9, 127.7, 127.1, 126.9, 126.1, 126.0, 125.9, 101.3 (Ph-CH), 88.64 (C-1), 81.4, 81.1, 74.8, 72.2, 70.7, 68.6, 21.2. $[\alpha]_D^{25}$ = −27.2° (c 0.5, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{31}H_{30}NaO_5S$ $[M + Na]^+$, 537.1712; found, 537.1709.

*p-Tolyl 2-O-Benzoyl-4,6-O-benzylidene-3-O-(naphthalene-2-ylmethyl)-1-thio-β-D-glucopyranoside (**11**).* A solution of **10** (8.6 g, 16.71 mmol), triethyl amine (5.8 mL, 41.77 mmol), benzoyl chloride (2.33 mL, 20.05 mmol), and a catalytic amount of *N*,*N*-dimethylaminopyridine in anhydrous CH_2Cl_2 (150 mL) was stirred at room temperature overnight. The reaction mixture was washed with saturated aqueous $NaHCO_3$ solution (3 × 150 mL), and the organic layer was dried over Na_2SO_4. The desired product **11** was obtained (9.9 g, 96%) after purification by flash column chromatography (hexanes/EtOAc/CH_2Cl_2 6:1:1) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 7.34 Hz, 2H), 7.71−7.68 (d, J = 8.80 Hz, 1H), 7.62−7.54 (m, 5H), 7.47−7.34 (m, 10H), 7.23 (d, J = 8.31 Hz, 1H), 7.10 (d, J = 7.83 Hz, 2H), 5.65 (s, 1H Ph-CH), 5.32 (dd, J = 9.78, 8.80 Hz, 1H), 4.99 (d, J = 12.23 Hz, 1H), 4.86 (d, J = 12.23 Hz, 1H), 4.78 (d, J = 9.78 Hz, 1H, H-1), 4.45 (dd, J = 10.76, 5.38 Hz, 1H), 3.95 (t, J = 8.80 Hz, 1H), 3.87 (t, J = 10.27 Hz, 1H), 3.86 (t, J = 9.29 Hz, 1H), 3.60−3.54 (m, 1H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.0, 138.5, 137.2, 135.2, 133.6, 133.2, 133.0, 132.9, 129.9, 129.8, 129.7, 129.1, 128.3, 128.3, 128.2, 128.0, 127.8, 127.6, 127.0, 126.2, 126.1, 125.9, 125.7, 101.3 (Ph-CH), 87.2 (C-1), 81.5, 79.1, 74.3, 72.0, 70.6, 68.6, 21.2. $[\alpha]_D^{25}$ = +19.5° (c 0.5, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{38}H_{34}NaO_6S$ $[M + Na]^+$, 641.1974; found, 641.1979.

General Procedure for Deprotection of 2-Naphthylmethyl Ethers. To the stirred solution of a 2-naphthylmethyl ether compound (1 mmol) in CH_2Cl_2 (18 mL) and water (1 mL) was added DDQ (2 mmol) at room temperature. After the reaction was stirred for 8 h, saturated aqueous $NaHCO_3$ solution was added, and the product was extracted with CH_2Cl_2. The combined organic layers were washed three times with saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4. After removal of the solvent in vacuum, the product was purified by silica gel chromatography.

*p-Tolyl 2-O-Benzoyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**12**).* Compound **12** (5.6 g, 92%) was prepared from **11** (7.88 g, 12.73 mmol) and DDQ (5.78 g, 25.47 mmol) according to the general procedure for deprotection of naphthylmethyl ethers and was purified by flash column chromatography (toluene/EtOAc 15:1 to 10:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (d, J = 8.24 Hz, 2H), 7.64−7.61 (m, 1H), 7.51−7.48 (m, 4H), 7.40−7.36 (m, 5H), 7.13 (d, J = 7.93 Hz, 2H), 5.56 (s, 1H Ph-CH), 5.14 (dd, J = 9.77, 8.85 Hz, 1H), 4.84 (d, J = 10.07 Hz, 1H, H-1), 4.43 (dd, J = 10.68, 4.88 Hz, 1H), 4.06−4.02 (m, 1H), 3.82 (t, J = 10.38 Hz, 1H), 3.62−3.53 (m, 2H), 2.83 (br s, 1H), 2.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9, 138.7, 136.8, 133.8, 133.5, 130.1, 129.8, 129.6, 129.4, 128.5, 128.4, 127.9, 126.3, 101.9 (Ph-CH), 86.8 (C-1), 80.6, 73.7, 73.3, 70.4, 68.5, 21.2. $[\alpha]_D^{25}$ = −45.2° (c 0.5, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{27}H_{26}NaO_6S$ $[M + Na]^+$, 501.1348; found, 501.1345.

General Procedure for Preactivation-Based Glycosylation Reactions. After the mixture of a glycosyl donor (1 mmol) and 4 Å MS (1.5 g) in CH_2Cl_2 (20 mL) was stirred at room temperature for 1 h, it was cooled to −78 °C, and AgOTf (3 mol in 6 mL dry acetonitrile) was added, followed by addition of *p*-toluene sulfenyl chloride (*p*-TolSCl) (1 mmol) via a microsyringe 10 min later. The mixture was stirred at −78 °C for an additional 15 min, when TLC showed that the donor was completely consumed. A solution of the acceptor (1 mmol) and 2,4,6-tri-*tert*-butyl pyrimidine (TTBP) (1 mmol) in CH_2Cl_2 (5 mL) was added. The resulting mixture was stirred for 20 min and warmed to room temperature, followed by filtration to remove MS. The filtrate was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated under vacuum. The resultant crude product was purified by silica gel flash column chromatography to get the desired compound.

*p-Tolyl [2-O-Benzoyl-4,6-O-benzylidene-3-O-(naphthalene-2-ylmethyl)-β-D-glucopyranosyl]-(1→3)-2-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**13**).* Compound **13** (6.15 g, 90%) was prepared from glycosyl donor **11** (4.37 g, 7.05 mmol) and acceptor **12** (3.375 g, 7.05 mmol) according to the general procedure for preactivation-based glycosylation and was purified by flash column chromatography (toluene/EtOAc 15:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (d, J = 7.32 Hz, 2H), 7.70 (d, J = 7.93 Hz, 1H), 7.58−7.48 (m, 8H), 7.46−7.33 (m, 12H), 7.30−7.16 (m, 6H), 7.08 (d, J = 7.93 Hz, 2H), 5.58 (s, 1H Ph-CH), 5.33 (s, 1H Ph-CH), 5.29 (t, J = 7.02 Hz, 1H), 5.28 (dd, J = 9.77, 9.16 Hz, 1H), 4.89 (d, J = 7.52 Hz, 1H H-1′), 4.87 (d, J = 12.51 Hz, 1H), 4.79 (d, J = 11.60 Hz, 1H), 4.78 (d, J = 10.07 Hz, 1H, H-1), 4.41 (dd, J = 10.38, 4.88 Hz, 1H), 4.21 (t, J = 9.16 Hz, 1H), 4.17 (dd, J = 10.68, 4.88 Hz, 1H), 3.95 (dd, J = 9.46, 9.16 Hz, 1H), 3.84 (t, J = 10.38 Hz, 1H), 3.81 (dd, J = 9.46, 9.16 Hz, 1H), 3.75 (dd, J = 8.55, 7.63 Hz, 1H), 3.70 (dd, J = 10.38, 10.07 Hz, 1H), 3.61−3.56 (m, 1H), 3.42−3.37 (m, 1H), 2.34 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.7, 164.6, 138.5, 137.3, 137.1, 135.3, 133.5, 133.0, 132.8, 132.7, 129.9, 129.7, 129.7, 129.5, 129.5, 129.4, 129.1, 129.0, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6, 126.7, 126.2, 126.1, 126.0, 125.8, 125.7, 101.6 (Ph-CH), 101.0 (Ph-CH), 100.5 (C-1′), 87.4 (C-1), 80.7, 79.3, 79.0, 78.2, 73.7, 73.4, 72.3, 70.7, 68.7, 68.6, 65.9, 21.2. $[\alpha]_D^{25}$ = +40.0° (c 0.5,

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

$CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{58}H_{52}NaO_{12}S$ $[M + Na]^+$, 995.3077; found, 995.3040.

*p-Tolyl [2-O-Benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl]-(1→3)-2-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**14**).* Compound **14** (1.63 g, 95%) was prepared from **13** (2.0 g, 2.05 mmol) and DDQ (0.93 g, 4.11 mmol) according to the general procedure for naphthylmethyl ether deprotection and purified by flash column chromatography (toluene/EtOAc 7:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.85 (d, J = 8.24 Hz, 2H), 7.70 (d, J = 8.24 Hz, 2H), 7.56−7.50 (m, 4H), 7.43−7.27 (m, 14H), 7.08 (d, J = 7.63 Hz, 2H), 5.57 (s, 1H Ph-CH), 5.33 (s, 1H Ph-CH), 5.26 (dd, J = 9.77, 8.85 Hz, 1H), 5.09 (dd, J = 7.63, 7.32 Hz, 1H), 4.93 (d, J = 7.02 Hz, 1H, H-1′), 4.79 (d, J = 10.07 Hz, 1H, H-1), 4.41 (dd, J = 10.38, 4.88 Hz, 1H), 4.25 (t, J = 8.85 Hz, 1H), 4.17 (dd, J = 10.38, 4.88 Hz, 1H), 3.86−3.77 (m, 3H), 3.70−3.66 (m, 2H), 3.61−3.56 (m, 1H), 3.39−3.34 (m, 1H), 2.63 (br s, 1H), 2.33 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.5, 164.7, 138.5, 137.1, 136.9, 133.5, 133.1, 133.0, 129.8, 129.8, 129.7, 129.5, 129.4, 129.3, 129.2, 128.3, 128.3, 128.2, 126.3, 126.2, 101.6 (Ph-CH), 101.56 (Ph-CH), 100.30 (C-1′), 87.36 (C-1), 80.4, 79.3, 79.1, 75.4, 72.6, 72.3, 70.7, 68.6, 68.6, 66.0, 21.2. HRMS (ESI-TOF) m/z: calcd for $C_{47}H_{44}NaO_{12}S$ $[M + Na]^+$, 855.2451; found, 855.2425.

*p-Tolyl [2-O-Benzoyl-4,6-O-benzylidene-3-O-(naphthalene-2-ylmethyl)-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-3-O-1-thio-β-D-glucopyranoside (**15**).* Compound **15** (3.3 g, 86%) was prepared from glycosyl donor **13** (2.23 g, 2.29 mmol) and acceptor **14** (1.91 g, 2.29 mmol) according to the general procedure for preactivation-based glycosylation and was purified by flash column chromatography (toluene/EtOAc 12:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.89 (d, J = 7.63 Hz, 2H), 7.82 (d, J = 7.93 Hz, 2H), 7.78 (d, J = 7.93 Hz, 2H), 7.71 (d, J = 7.93 Hz, 1H), 7.65 (d, J = 7.63 Hz, 2H), 7.59−7.44 (m, 11H), 7.43−7.25 (m, 25H), 7.23−7.19 (m, 4H), 7.09 (d, J = 7.93 Hz, 2H), 5.55 (s, 1H Ph-CH), 5.46 (s, 1H Ph-CH), 5.35 (dd, J = 7.93, 7.63 Hz, 1H), 5.19 (dd, J = 5.80, 5.49 Hz, 1H), 5.01 (d, J = 6.41 Hz, 1H anomeric), 4.99 (d, J = 7.93 Hz, 1H anomeric), 4.97 (s, 1H), 4.92 (d, J = 12.21 Hz, 1H), 4.87−4.79 (m, 4H, 1H anomeric), 4.69 (d, J = 10.07 Hz, 1H, H-1), 4.63 (s, 1H Ph-CH), 4.37 (dd, J = 10.38, 4.58 Hz, 1H), 4.23 (dd, J = 10.38, 4.58 Hz, 1H), 4.16−4.07 (m, 4H), 3.97 (dd, J = 8.24, 7.93 Hz, 1H), 3.93−3.90 (m, 2H), 3.85 (dd, J = 8.85, 8.24 Hz, 1H), 3.77−3.71 (m, 2H), 3.62−3.57 (m, 3H), 3.49−3.41 (m, 4H), 3.27 (dd, J = 9.46, 9.16 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.0, 164.8, 164.6, 164.6, 138.3, 137.4, 137.3, 137.3, 137.9, 135.4, 133.5, 133.3, 133.2, 133.1, 133.1, 132.9, 132.8, 129.8, 129.8, 129.8, 129.7, 129.7, 129.6, 129.5, 129.4, 129.4, 129.1, 129.0, 129.0, 128.9, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.6, 126.7, 126.4, 126.3, 126.1, 126.0, 125.8, 125.6, 102.0, 101.1, 100.9, 100.9, 99.3 (anomeric), 98.0 (anomeric), 97.0 (anomeric), 87.9 (C-1), 81.3, 78.6, 78.5, 78.2, 77.1, 75.7, 74.3, 73.7, 73.5, 73.2, 73.0, 72.7, 70.7, 68.7, 68.7, 68.5, 66.0, 65.7, 65.4, 21.2. $[\alpha]_D^{25}$ = +43.0° (c 0.5, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{98}H_{89}O_{24}S$ $[M + H]^+$, 1681.5465; found, 1681.5461.

*2-Azidoethyl [2-O-Benzoyl-4,6-O-benzylidene-β-D-glucopyranosyl]-(1→3)-2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (**16**).* The reaction between **13** (3.5 g, 3.60 mmol) and 2-azidoethanol (0.5 g, 5.65 mmol) was carried out according to the general procedure for preactivation-based glycosylation, and the crude product was directly subjected to deprotection by the general procedure to remove the naphthylmethyl group to afford **16** (2.5 g, 91%), which was purified by flash column chromatography (toluene/EtOAc 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, J = 7.83 Hz, 2H), 7.74 (d, J = 7.83 Hz, 2H), 7.55−7.49 (m, 4H), 7.43−7.24 (m, 12H), 5.56 (s, 1H Ph-CH), 5.36 (s, 1H Ph-CH), 5.27 (dd, J = 8.31, 7.34 Hz, 1H), 5.12 (dd, J = 8.31, 7.34 Hz, 1H), 4.95 (d, J = 6.85 Hz, 1H, H-1′), 4.67 (d, J = 7.34 Hz, 1H, H-1), 4.37 (dd, J = 10.27, 4.89 Hz, 1H), 4.24−4.19 (m, 2H), 3.88−3.79 (m, 4H), 3.70 (dd, J = 10.76, 10.27 Hz, 1H), 3.66 (t, J = 9.29 Hz, 1H), 3.61−3.49 (m, 2H), 3.43−3.37 (m, 1H), 3.28−3.18 (m, 2H), 2.60 (br d, J = 3.91 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 164.6, 137.1, 136.9, 133.1, 133.0, 129.8, 129.7, 129.3, 129.3, 129.2, 129.0, 128.3, 128.3, 128.2, 126.3, 126.1, 101.7 (Ph-CH), 101.4 (Ph-CH), 101.1 (C-1), 100.29 (C-1′), 80.5, 79.1, 77.8, 75.2, 73.2, 72.6, 68.7, 68.6, 67.7, 66.5, 66.0, 50.6. $[\alpha]_D^{25}$ = −22.0° (c 1, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{42}H_{42}N_3O_{13}$ $[M + H]^+$, 796.2718; found, 796.2697.

*2-Azidoethyl 3-O-[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 (**17**).* Compound **17** (1.53 g, 90%) was prepared from glycosyl donor **13** (1.10 g, 1.13 mmol) and acceptor **16** (0.905 g, 1.13 mmol) according to the general procedure for preactivation-based glycosylation and was purified by flash column chromatography (toluene/EtOAc 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, J = 7.83 Hz, 2H), 7.87 (d, J = 7.83 Hz, 2H), 7.79 (d, J = 7.83 Hz, 2H), 7.67 (d, J = 7.83 Hz, 2H), 7.57−7.44 (m, 7H), 7.42−7.30 (m, 17H), 7.28−7.18 (m, 8H), 5.54 (s, 1H), 5.42 (s, 1H), 5.18 (t, J = 7.34 Hz, 1H), 5.17 (t, J = 8.80 Hz, 1H), 5.05 (d, J = 7.83 Hz, 1H anomeric), 5.00 (d, J = 5.38 Hz, 1H anomeric), 4.94 (dd, J = 8.31, 7.83 Hz, 1H), 4.87 (s, 1H), 4.83 (t, J = 8.80 Hz, 1H), 4.82 (d, J = 5.38 Hz, 1H anomeric), 4.76 (s, 1H), 4.56 (d, J = 7.83 Hz, 1H anomeric), 4.34 (dd, J = 10.27, 4.40 Hz, 1H), 4.21 (dd, J = 10.76, 4.89 Hz, 1H), 4.15−4.06 (m, 4H), 4.00−3.92 (m, 3H), 3.89−3.84 (m, 1H), 3.76−3.63 (m, 3H), 3.60−3.42 (m, 8H), 3.37 (t, J = 9.29 Hz, 1H), 3.30−3.20 (m, 2H), 2.66 (br d, J = 3.42 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 164.7, 164.6, 164.6, 137.3, 137.2, 137.1, 137.0, 133.6, 133.4, 133.2, 133.1, 129.9, 129.8, 129.7, 129.7, 129.4, 129.3, 129.3, 129.1, 128.9, 128.6, 128.6, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 126.4, 126.3, 126.3, 126.1, 125.3, 101.8, 101.8, 101.3, 101.1, 100.8 (anomeric), 98.8 (anomeric), 98.4 (anomeric), 97.0 (anomeric), 80.8, 78.7, 78.3, 77.5, 76.9, 75.0, 74.8, 74.4, 73.7, 73.4, 72.6, 72.5, 68.7, 68.6, 67.9, 66.5, 66.0, 65.5, 50.6. $[\alpha]_D^{25}$ = +6.0° (c 0.5, $CHCl_3$). HRMS (ESI-TOF) m/z: calcd for $C_{82}H_{81}N_4O_{25}$ $[M + Na]^+$, 1521.5190; found, 1521.5153.

*2-Azidoethyl [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-glucopyranosyl]-(1→3)-2-O-benzoyl-4,6-O-benzylidene-β-D-glucopyranoside (**18**).* Compound **18** (0.606 g, 87%) was prepared from glycosyl donor **13** (0.306 g, 0.315 mmol) and acceptor **17** (0.475 g, 0.315 mmol) by the same synthetic procedure as that for **17** and was purified by flash column chromatography (toluene/EtOAc 10:1 to 6:1). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.95 (d, J = 7.2 Hz, 2H), 7.86 (d, J = 7.3 Hz,

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

2H), 7.81−7.77 (m, 5H), 7.65 (d, *J* = 7.3 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.51 (m, 2H), 7.47−7.17 (m, 45H), 5.52 (s, 1H), 5.43 (s, 1H), 5.22−5.17 (m, 2H), 5.10 (d, *J* = 7.5 Hz, 1H), 5.03−4.96 (m, 2H), 4.94−4.85 (m, 6H), 4.79 (m, 4H), 4.55 (d, *J* = 7.6 Hz, 1H), 4.33 (dd, *J* = 10.4, 4.8 Hz, 1H), 4.22 (dd, *J* = 10.3, 4.9 Hz, 1H), 4.18−4.07 (m, 6H), 4.00 (m, 4H), 3.92 (dd, *J* = 6.8, 4.4 Hz, 1H), 3.88−3.84 (m, 1H), 3.69 (m, 3H), 3.60 (m, 1H), 3.56−3.38 (m, 12H), 3.33−3.27 (m, 2H), 3.23 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.86, 164.84, 164.6, 164.5, 137.4, 137.27, 137.25, 137.1, 137.0, 133.8, 133.6, 133.56, 133.5, 133.2, 133.1, 129.9, 129.74, 129.7, 129.6, 129.5, 129.4, 129.34, 129.31, 129.23, 129.17, 129.2, 129.1, 129.0, 128.97, 128.8, 128.7, 128.6, 128.58, 128.55, 128.4, 128.3, 128.27, 128.2, 128.1, 127.99, 126.4, 126.36, 126.33, 126.3, 126.1, 125.3, 101.9, 101.8, 101.3, 101.24, 101.21, 101.19, 100.7, 98.43, 98.39, 97.2, 97.1, 96.9, 80.8, 79.1, 78.7, 78.1, 77.9, 77.88, 77.5, 76.6, 75.0, 74.8, 74.6, 74.3, 73.7, 73.4, 73.2, 72.9, 72.5, 72.4, 68.7, 68.6, 67.9, 66.5, 66.0, 65.6, 65.5, 65.48, 50.6. $[\alpha]_D^{25}$ = +22.0° (*c* 0.5, $CHCl_3$). MS (MALDI TOF) *m*/*z*: calcd. For $C_{122}H_{114}N_3O_{37}$ $[M + H]^+$, 2212.7131; found, 2212.7119.

*2-Azidoethyl [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-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 (**19**).* Compound **19** (0.74 g, 81%) was prepared from glycosyl donor **15** (0.53 g, 0.315 mmol) and acceptor **17** (0.47 g, 0.315 mmol) by the same synthetic procedure as that for **17** and purified by flash column chromatography (toluene/EtOAc 8:1 to 4:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, *J* = 6.85 Hz, 2H), 7.87−7.77 (m, 12H), 7.66 (d, *J* = 7.83 Hz, 2H), 7.58 (d, *J* = 7.34 Hz, 2H), 7.55−7.16 (m, 62H), 5.51 (s, 1H), 5.45 (s, 1H), 5.22 (t, *J* = 7.83 Hz, 1H), 5.21 (t, *J* = 8.31 Hz, 1H), 5.12 (d, *J* = 7.83 Hz, 1H), 5.02 (d, *J* = 4.89 Hz, 1H), 4.94−4.79 (m, 16H), 4.77 (d, *J* = 8.31 Hz, 1H), 4.55 (d, *J* = 7.34 Hz, 1H), 4.33 (dd, *J* = 10.76, 4.89 Hz, 1H), 4.23 (dd, *J* = 10.27, 4.89 Hz, 1H), 4.16−4.08 (m, 8H), 4.04−3.96 (m, 6H), 3.91−3.84 (m, 2H), 3.75−3.67 (m, 3H), 3.62−3.34 (m, 19H), 3.32−3.23 (m, 4H), 2.61 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 164.9, 164.8, 164.7, 164.7, 164.7, 164.6, 137.4, 137.4, 137.4, 137.3, 137.2, 137.1, 133.8, 133.8, 133.7, 133.7, 133.9, 133.7, 133.64, 133.63, 133.56, 133.55, 133.22, 133.20, 133.19, 123.0, 129.8, 129.7, 129.66, 129.56, 129.44, 129.41, 129.34, 129.27, 129.25, 129.2, 129.13, 129.09, 129.06, 129.0, 128.9, 128.7, 128.6, 128.58, 128.44, 128.4, 128.33, 128.30, 128.2, 128.1, 128.05, 126.5, 126.48, 126.42, 126.38, 126.2, 125.4, 102.0, 101.8, 101.4, 101.39, 101.32, 101.27, 101.2, 100.7, 98.5, 98.32, 97.2, 97.2, 97.14, 97.05, 97.0, 96.9, 80.9, 79.0, 78.7, 78.1, 78.0, 77.9, 77.9, 76.6, 74.9, 74.8, 74.6, 74.5, 74.4, 74.3, 74.2, 73.8, 73.5, 73.2, 73.12, 73.11, 73.05, 72.95, 72.94, 72.5, 68.8, 68.7, 68.66, 68.6, 68.0, 66.5, 66.1, 65.6, 65.58, 65.51, 50.7. $[\alpha]_D^{25}$ = +27.0° (*c* 0.5, $CHCl_3$). MS (MALDI TOF) *m*/*z*: calcd for $C_{162}H_{149}N_3NaO_{49}$ $[M + Na]^+$, 2944.89; found, 2944.71.

*2-Azidoethyl [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-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-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 (**20**).* Compound **20** (0.156 g, 80%) was prepared from glycosyl donor **13** (108 mg, 0.11 mmol) and acceptor **19** (158 mg, 0.05 mmol) by the same synthetic procedure as that for **17** and was purified by flash column chromatography (toluene/EtOAc 10:1 to 4:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.99 (d, *J* = 7.63 Hz, 2H), 7.89−7.79 (m, 15H), 7.68 (d, *J* = 7.93 Hz, 2H), 7.61 (d, *J* = 7.63 Hz, 2H), 7.57−7.17 (m, 79H), 5.53 (s, 1H), 5.46 (s, 1H), 5.24 (t, *J* = 7.93 Hz, 1H), 5.23 (t, *J* = 7.93 Hz, 1H), 5.15 (d, *J* = 7.63 Hz, 1H), 5.05 (d, *J* = 4.58 Hz, 1H), 5.00−4.77 (m, 23H), 4.56 (d, *J* = 7.63 Hz, 1H), 4.35 (dd, *J* = 9.77, 4.27 Hz, 1H), 4.25 (dd, *J* = 10.38, 4.58 Hz, 1H), 4.18−4.09 (m, 10H), 4.07−3.96 (m, 8H), 3.94−3.86 (m, 2H), 3.76−3.68 (m, 3H), 3.66−3.41 (m, 22H), 3.40−3.23 (m, 7H), 2.64 (br s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9, 164.9, 164.8, 164.77, 164.75, 164.72, 164.70, 164.68, 164.5, 137.8, 137.4, 137.36, 137.34, 137.31, 137.2, 137.1, 133.8, 133.7, 133.6, 133.58, 133.52, 133.19, 133.16, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.37, 129.33, 129.23, 129.19, 129.17, 129.1, 128.8, 128.7, 128.65, 128.58, 128.4, 128.3, 128.26, 128.18, 128.14, 128.1, 128.0, 126.5, 126.39, 126.36, 126.33, 126.1, 125.3, 105.0, 102.0, 101.8, 101.4, 101.33, 101.29, 101.26, 101.2, 101.1, 100.6, 98.4, 98.2, 97.2, 97.1, 97.02, 96.98(2C), 96.91, 96.86, 96.8, 80.9, 78.7, 78.1, 78.0, 77.94, 77.91, 77.88, 77.8, 77.5, 76.6, 74.8, 74.5, 74.4, 74.4, 74.2, 74.1, 73.8, 73.5, 73.1, 73.0, 72.95, 72.9, 72.6, 72.4, 68.7, 68.6, 68.0, 66.48, 66.06, 65.60, 65.55, 65.50, 65.47, 50.67. $[\alpha]_D^{25}$ = +31.2° (*c* 0.5, $CHCl_3$). MS (MALDI TOF) *m*/*z*: calcd for $C_{202}H_{185}N_3NaO_{61}$ $[M + Na]^+$, 3651.13; found, 3651.50.

*2-Azidoethyl [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-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-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 (**21**).* Compound **21** (0.380 g, 85%) was prepared from glycosyl donor **15** (218 mg, 0.13 mmol) and acceptor **19** (300 mg, 0.10 mmol) by the same synthetic procedure as that for **17** and was purified by flash column chromatography (toluene/EtOAc 12:1 to 3:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.97 (d, *J* = 7.02 Hz, 2H), 7.87−7.77 (m, 17H), 7.66 (d, *J* = 7.32 Hz, 2H), 7.59 (d, *J* = 7.32 Hz, 2H), 7.55−7.15 (m, 97H), 5.51 (s, 1H), 5.45 (s, 1H), 5.22 (t, *J* = 8.55 Hz, 1H), 5.21 (t, *J* = 7.32 Hz, 1H), 5.13 (d, *J* = 7.63 Hz, 1H), 5.02 (d, *J* = 5.19 Hz, 1H), 4.92−4.73 (m, 29H), 4.55 (d, *J* = 7.63 Hz, 1H), 4.33 (dd, *J* = 10.38, 4.88 Hz, 1H), 4.24 (dd, *J* = 10.38, 4.88 Hz, 1H), 4.16−4.07 (m, 11H), 4.04−3.95 (m, 10H), 3.91−3.84 (m, 2H), 3.75−3.67 (m, 3H), 3.64−3.34 (m, 30H), 3.33−3.20 (m, 7H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.9, 164.9, 164.8, 164.79, 164.78, 164.77, 164.76, 164.74, 164.72, 164.7, 164.6, 164.5, 137.9, 137.4, 137.33, 137.30, 137.2, 137.0, 133.8, 133.7, 133.6, 133.2, 129.9, 129.8, 129.7, 129.6, 129.5, 129.38, 129.35, 129.3, 129.23, 129.2, 129.1, 129.0, 128.8, 128.7, 128.6, 128.4, 128.3, 128.2, 128.18, 128.13, 128.08, 127.9,

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

126.5, 126.4, 126.35, 126.32, 126.1, 125.3, 102.0, 101.8, 101.4, 101.33, 101.30, 101.26, 101.24, 101.22, 101.20, 101.18, 101.1, 100.6, 98.4, 98.2, 97.1, 97.12, 97.09, 96.98 (2C), 96.91, 96.88, 96.85, 96.84, 96.8, 80.9, 78.7, 78.1, 78.0, 77.96, 77.9, 77.88, 77.8, 77.5, 77.3, 76.59, 76.58, 74.8, 74.5, 74.4, 74.3, 74.2, 73.8, 73.5, 73.2, 73.1, 73.0, 72.9, 72.8, 72.6, 72.4, 68.8, 68.7, 68.67, 68.62, 68.0, 66.5, 66.1, 65.6, 65.6, 65.5, 50.7. $[\alpha]_D^{25}$ = +36.0° (*c* 0.5, $CHCl_3$). MS (MALDI TOF) *m*/*z*: calcd for $C_{242}H_{221}N_3NaO_{73}$ $[M + H]^+$, 4359.35; found, 4360.53.

General Procedure for Global Deprotection of 18−21. To a solution of **18**, **19**, **20**, or **21** (23 μmol) in CH_2Cl_2 (12 mL) were added acetic acid (8 drops) and zinc powder (80 mg). The mixture was vigorously stirred at room temperature for 24 h and then filtered through a pad of Celite. The filtrate was condensed in vacuum, and the resulting residue was dissolved in AcOH and H_2O (5:1, 60 mL) and heated at 60 °C for 24 h. The solvents were removed in vacuum, and the residue was coevaporated with toluene 5 times. After the product was dissolved in *t*-BuOH and H_2O (4:1, 60 mL), NaOH (120 mg) in H_2O (6.0 mL) was added in portions. The mixture was stirred at 40 °C for 24 h, neutralized with 0.25 N HCl at 0 °C, and lyophilized. The crude product was purified on a sephadex G-25 gel filtration column using water as the eluent, and the product fractions were lyophilized to afford the desired free oligosaccharides.

*2-Aminoethyl β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-β-D-glucopyranoside (**22**).* Compound **22** (18.7 mg) was prepared from **18** (50.0 mg, 23 μmol) by the above general procedure in 80% yield. ^{1}H NMR (600 MHz, D_2O): δ 4.59 (m, 5H), 4.39 (d, *J* = 8.1 Hz, 1H), 3.97 (dd, *J* = 10.9, 5.1 Hz, 1H, 1/2O$\underline{CH_2}CH_2$), 3.85−3.74 (m, 6H), 3.68−3.55 (m, 10H), 3.47−3.30 (m, 13H), 3.29−3.16 (m, 4H), 3.15−3.09 (m, 2H, CH_2N_3). ^{13}C NMR (150 MHz, D_2O): δ 102.7, 102.5, 101.8, 84.2, 84.0, 76.0, 75.6, 75.5, 73.4, 73.2, 72.7, 69.5, 68.0, 65.8, 60.6, 60.5, 60.03, 60.02, 39.4. HRMS (ESI-TOF,) *m*/*z*: calcd for $C_{50}H_{88}NO_{41}$ $[M + H]^+$, 1304.3775; found, 1034.3727.

*2-Aminoethyl β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**23**).* Compound **23** (10.2 mg) was prepared from **19** (25 mg, 8.5 μmol) by the above general procedure in 88% yield. ^{1}H NMR (600 MHz, D_2O): δ 4.57 (m, 7H), 4.35 (d, *J* = 8.0 Hz, 1H), 3.93 (m, 1H), 3.78−3.71 (m, 8H), 3.62−3.50 (m, 16H), 3.40−3.26 (m, 22H), 3.19 (m, 3H), 3.08 (t, *J* = 4.9 Hz, 2H). ^{13}C NMR (150 MHz, D_2O): δ 102.7, 102.4, 101.8, 84.1, 84.1, 84.0, 75.9, 75.5, 75.5, 73.4, 73.2, 73.1, 72.7, 69.5, 68.0, 67.9, 65.8, 60.6, 60.4, 39.3. HRMS (ESI-TOF) *m*/*z*: calcd for $C_{50}H_{88}NO_{41}$ $[M + H]^+$, 1358.4832; found, 1358.4775.

*2-Aminoethyl β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-(1→3)-β-D-glucopyranoside (**24**).* Compound **24** (11.0 mg) was prepared from **20** (27.8 mg, 7.6 μmol) by the above general procedure in 85% yield. ^{1}H NMR (600 MHz, D_2O): δ 4.66−4.59 (m, 9H), 4.39 (d, *J* = 8.24 Hz, 1H), 4.00−3.96 (m, 1H), 3.82−3.74 (m, 12H), 3.65−3.55 (m, 19H), 3.42−3.31 (m, 26H), 3.27−3.19 (m, 4H), 3.13−3.11 (m, 2H). ^{13}C NMR (150 MHz, D_2O): δ 102.7, 102.5, 101.8, 84.1, 84.0, 75.9, 75.6, 73.4, 73.2, 72.7, 71.6, 69.5, 69.4, 68.0, 65.78, 60.6, 60.3, 60.0, 39.4. HRMS (ESI-TOF) *m*/*z*: calcd for $C_{62}H_{108}NO_{51}$ $[M + H]^+$, 1682.5888; found, 1682.5787.

*2-Aminoethyl β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl]-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**25**).* Compound **25** (10.4 mg) was prepared from **21** (25.6 mg, 5.9 μmol) by the above general procedure in 88% yield. ^{1}H NMR (600 MHz, D_2O): δ 4.65−4.59 (m, 11H), 4.39 (d, *J* = 7.83 Hz, 1H), 3.98−3.93 (m, 1H), 3.80−3.74 (m, 13H), 3.65−3.54 (m, 24H), 3.42−3.31 (m, 32H), 3.27−3.18 (m, 4H), 3.08−3.06 (m, 2H). ^{13}C NMR (151 MHz, D_2O): δ 102.7, 102.4, 101.8, 84.1, 84.1, 83.9, 75.9, 75.5, 73.4, 73.2, 72.7, 71.6, 69.6, 69.5, 69.3, 68.0, 65.8, 64.8, 62.4, 60.6, 60.4, 60.3, 60.0, 39.3. HRMS (ESI-TOF) *m*/*z*: calcd for $C_{74}H_{128}NNaO_{61}$ $[M + H + Na]^{2+}$, 1014.8421; found, 1014.8417.

General Procedure for Activation of Amino Oligosaccharides 22−25. Each oligosaccharide was dissolved in DMF and 0.1 M PBS buffer (4:1, 0.5 mL), and then disuccinimidal glutarate (15 equiv) was added to the solution. The reaction was kept under gentle stirring at room temperature for 4 h, followed by removal of the solvents under vacuum. The excessive reagent was removed from the reaction by precipitation with 9 volumes of EtOAc, and the precipitates were washed 10 times with EtOAc followed by drying under vacuum to give activated oligosaccharides **26−29**.

General Procedure for Conjugating Activated Oligosaccharides 26−29 with KLH and HSA. After solutions of **26−29** and KLH or HSA in a molar ratio of 30:1 in 0.1 M PBS buffer (0.35 mL) were gently stirred at room temperature for 3 days, they were applied to a Biogel A0.5 column to separate the resultant glycoconjgates **1−8** from unreacted oligosaccharides using 0.1 M PBS buffer (*I* = 0.1, pH 7.8) as the eluent. Fractions containing glycoconjugates, which were confirmed by the bicinchoninic acid (BCA) assay for protein and the phenol−sulfuric acid assay for carbohydrate, were combined and dialyzed against distilled water for 2 days. The solutions were lyophilized to obtain **1−8** as white solids.

Analysis of the Carbohydrate Loadings of Glycoconjugates 1−8.[32] Aliquots of a standard D-glucose solution (1 mg/mL) in water were added in ten dry 10 mL test tubes in 5 μL increments to form standard samples that contained 0−50 μg of glucose. In another test tube, an accurately weighed sample of to-be-analyzed glycoconjugate **1−8** was placed. The content of glucose in each tested sample was estimated to be also in the range of 0−50 μg. To all of the tubes were then added 500 μL of 4% phenol and 2.5 mL of 96% sulfuric acid at room temperature. About 20 min later, the resultant colored solutions were transferred into cuvettes, and their absorption at 490 nm wavelength (A_{490}) was measured. A sugar calibration curve was created by plotting the A_{490} values of standard samples against their glucose content (in micrograms), which was employed to calculate the glucose content of each tested glycoconjugate sample based on its A_{490} value. The carbohydrate loading of each glycoconjugate was calculated according to the following equation, and the results are shown in Table 1.

$$\text{carbohydrate loading \%} = \frac{\text{sugar weight in a tested sample}}{\text{total weight of the sample}} \times 100\%$$

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

Immunization of Mouse. After each glycoconjugate (2.17 mg of **1**, 2.32 mg of **3**, 2.40 mg of **5**, or 1.98 mg of **7**) was dissolved in 0.3 mL of 10× PBS buffer, it was diluted with water to form a 2× PBS solution (1.5 mL). The solution was well-mixed with 1.5 mL of Titermax Gold adjuvant (1:1, v/v) to form an emulsion according to the protocol given by the manufacturer. Each group of six female C57BL/6J mice was initially immunized (day 1) by i.m. injection of 0.1 mL of the emulsion described above. Following the initial immunization, mice were boosted 4 times on days 14, 21, 28, and 38 by s.c. injection of the same conjugate emulsion. Therefore, each injected dose of glycoconjugate contained about 6 μg of the carbohydrate antigen. Mouse blood samples were collected through the leg veins of each mouse on day 0 prior to the initial immunization and on days 27, 38, and 48 after the boost immunizations. Finally, antisera were obtained from the clotted blood samples and stored at −80 °C before use.

The ELISA Protocol. Each well in the ELISA plate was treated with 100 μL of a solution of an individual HSA conjugate **5**, **6**, **7**, or **8** (2 μg/mL) dissolved in coating buffer (0.1 M bicarbonate, pH 9.6) at 4 °C overnight and then at 37 °C for 1 h, which was followed by washing (3 times) with PBS buffer containing 0.05% Tween-20 (PBST) and treatment with blocking buffer (10% BSA in PBS buffer containing NaN_3) at room temperature for 1 h. After 3 times of washing with PBST, half-log serially diluted solutions (from 1:300 to 1:656 100) of a pooled or an individual mouse antiserum in PBS were added to the coated ELISA plates (100 μL/well), followed by incubation at 37 °C for 2 h. The plates were then washed with PBST and incubated at room temperature for another 1 h with a 1:1000 diluted solution of alkaline phosphatase (AP)-linked goat anti-mouse kappa, IgG1, IgG2a, or IgM antibody (100 μL/well), respectively. Finally, the plates were washed with PBST and developed with 100 μL of *p*-nitrophenylphosphate (PNPP) solution (1.67 mg/mL in buffer) for 30 min at room temperature, which was followed by colorimetric readout at 405 nm using a microplate reader. The optical density (OD) values were plotted against the logarithmic scale of antiserum dilution values, and a best-fit line was obtained. The equation of the line was employed to calculate the dilution value at which an OD of 0.2 was achieved, and the antibody titer was obtained as the inverse of the dilution value.

***In Vivo* Evaluation of the New Vaccine 2 To Elicit Protection against Fungal Infection.** A group of 11 female C57BL/6J mice were immunized with an emulsion of conjugate **2** (containing 6 μg of octasaccharide antigen per dose) and Titermax Gold adjuvant prepared according to the protocol described above or with PBS (control group) on days 1, 14, 21, and 28. Thereafter, *C. albicans* (strain SC5314) cells (7.5×10^5 per mouse) in 200 μL of PBS were injected in the mice by i.v. administration on day 38. *C. albican* cells used in this experiment were cultured in YEPD medium at 28 °C for 24 h, and before injection, they were centrifuged and washed 3 times with PBS. The mice were checked on a daily basis, and the observation continued for 32 days after the injection of *C. albican* cells. Note that the animal protocols for the immunization and fungal challenge experiments were approved by the Institutional Animal Use and Care Committees of Wayne State University and Second Military Medical University.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Calculated antibody titers from various ELISA experiments and NMR and MS spectra of synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: zwguo@chem.wayne.edu.

Author Contributions

‖G.L. and Z.Z. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported in part by an NIH/NCI grant (R01 CA95142) and by the National Major Scientific and Technological Special Project for "Significant New Drugs Development" of China (grant no. 2012ZX09502001-005) and the National High Technology Research and Development Program of China (grant no. 2012AA021500). The authors thank Dr. B. Ksebati for performing some of the 2D NMR measurements. The 600 MHz NMR instrument used in this study was financed by an NSF grant (CHE-0840413).

■ REFERENCES

(1) Limper, A. H., Knox, K. S., Sarosi, G. A., Ampel, N. M., Bennett, J. E., Catanzaro, A., Davies, S. F., Dismukes, W. E., Hage, C. A., Marr, K. A., Mody, C. H., Perfect, J. R., and Stevens, D. A. (2011) An official American Thoracic Society statement: Treatment of fungal infections in adult pulmonary and critical care patients. *Am. J. Respir. Crit. Care Med. 183*, 96−128.

(2) Wisplinghoff, H., Bischoff, T., Tallent, S. M., Seifert, H., Wenzel, R. P., and Edmond, M. B. (2004) Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin. Infect. Dis. 39*, 309−317.

(3) Pfaller, M. A., and Diekema, D. J. (2007) Epidemiology of invasive candidiasis: a persistent public health problem. *Clin. Microbiol. Rev. 20*, 133−163.

(4) Johnson, M. A., and Bundle, D. R. (2013) Designing a new antifungal glycoconjugate vaccine. *Chem. Soc. Rev. 42*, 4327−4344.

(5) Cassone, A. (2013) Development of vaccines for *Candida albicans*: fighting a skilled transformer. *Nat. Rev. Microbiol. 11*, 884−891.

(6) Cutler, J. E., Deepe, G. S., Jr., and Klein, B. S. (2007) Advances in combating fungal diseases: vaccines on the threshold. *Nat. Rev. Microbiol. 5*, 13−28.

(7) Bowman, S. M., and Free, S. J. (2006) The structure and synthesis of the fungal cell wall. *BioEssays 28*, 799−808.

(8) Bromuro, C., Torosantucci, A., Chiani, P., Conti, S., Polonelli, L., and Cassone, A. (2002) Interplay between protective and inhibitory antibodies dictates the outcome of experimentally disseminated candidiasis in recipients of a *Candida albicans* vaccine. *Infect. Immun. 70*, 5462−5470.

(9) Torosantucci, A., Bromuro, C., Chiani, P., De Bernardis, F., Berti, F., Galli, C., Norelli, F., Bellucci, C., Polonelli, L., Costantino, P., Rappuoli, R., and Cassone, A. (2005) A novel glyco-conjugate vaccine against fungal pathogens. *J. Exp. Med. 202*, 597−606.

(10) Torosantucci, A., Chiani, P., Bromuro, C., De Bernardis, F., Palma, A. S., Liu, Y., Mignogna, G., Maras, B., Colone, M., Stringaro, A., Zamboni, S., Feizi, T., and Cassone, A. (2009) Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. *PLoS One 4*, e5392.

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476

(11) Bromuro, C., Romano, M., Chiani, P., Berti, F., Tontini, M., Proietti, D., Mori, E., Torosantucci, A., Costantino, P., Rappuoli, R., and Cassone, A. (2010) Beta-glucan-CRM197 conjugates as candidates antifungal vaccines. *Vaccine 28*, 2615−2623.
(12) Hu, Q.-Y., Allan, M., Adamo, R., Quinn, D., Zhai, H., Wu, G., Clark, K., Zhou, J., Ortiz, S., Wang, B., Danieli, E., Crotti, S., Tontini, M., Brogioni, G., and Berti, F. (2013) Synthesis of a well-defined glycoconjugate vaccine by a tyrosine-selective conjugation strategy. *Chem. Sci. 4*, 3827.
(13) Adamo, R., Hu, Q.-Y., Torosantucci, A., Crotti, S., Brogioni, G., Allan, M., Chiani, P., Bromuro, C., Quinn, D., Tontini, M., and Berti, F. (2014) Deciphering the structure−immunogenicity relationship of anti-Candida glycoconjugate vaccines. *Chem. Sci. 5*, 4302−4311.
(14) Adamoa, R., Tontinia, M., Brogionia, G., Romanoa, M. R., Costantinia, G., Danielia, E., Proiettia, D., Bertia, F., and Costantinoa, P. (2011) Synthesis of laminarin fragments and evaluation of a *β*-(1,3) glucan hexasaccaride-CRM197 conjugate as vaccine candidate against *Candida albicans*. *J. Carbohydr. Chem. 30*, 249−280.
(15) Verez-Bencomo, V., Fernandez-Santana, V., Hardy, E., Toledo, M. E., Rodriguez, M. C., Heynngnezz, L., Rodriguez, A., Baly, A., Herrera, L., Izquierdo, M., Villar, A., Valdes, Y., Cosme, K., Deler, M. L., Montane, M., Garcia, E., Ramos, A., Aguilar, A., Medina, E., Torano, G., Sosa, I., Hernandez, I., Martinez, R., Muzachio, A., Carmenates, A., Costa, L., Cardoso, F., Campa, C., Diaz, M., and Roy, R. (2004) A synthetic conjugate polysaccharide vaccine against Haemophilus influenzae type b. *Science 305*, 522−525.
(16) Yu, H., Williams, D. L., and Ensley, H. E. (2005) 4-Acetoxy-2,2-dimethylbutanoate: a useful carbohydrate protecting group for the selective formation of beta-(1→3)-D-glucans. *Tetrahedron Lett. 46*, 3417−3421.
(17) Jamois, F., Ferrieres, V., Guegan, J. P., Yvin, J. C., Plusquellec, D., and Vetvicka, V. (2005) Glucan-like synthetic oligosaccharides: iterative synthesis of linear oligo-beta-(1,3)-glucans and immunostimulatory effects. *Glycobiology 15*, 393−407.
(18) Zeng, Y., Ning, J., and Kong, F. (2003) Remote control of alpha- or beta-stereoselectivity in (1→3)-glucosylations in the presence of a C-2 ester capable of neighboring-group participation. *Carbohydr. Res. 338*, 307−311.
(19) Mo, K. F., Li, H., Mague, J. T., and Ensley, H. E. (2009) Synthesis of the beta-1,3-glucan, laminarahexaose: NMR and conformational studies. *Carbohydr. Res. 344*, 439−447.
(20) Weishaupt, M. W., Matthies, S., and Seeberger, P. H. (2013) Automated solid-phase synthesis of a beta-(1,3)-glucan dodecasaccharide. *Chemistry 19*, 12497−12503.
(21) Tanaka, H., Kawai, T., Adachi, Y., Ohno, N., and Takahashi, T. (2010) Beta(1,3) branched heptadeca- and linear hexadeca-saccharides possessing an aminoalkyl group as a strong ligand to dectin-1. *Chem. Commun. 46*, 8249−8251.
(22) Anish, C., Schumann, B., Pereira, C. L., and Seeberger, P. H. (2014) Chemical biology approaches to designing defined carbohydrate vaccines. *Chem. Biol. 21*, 38−50.
(23) Yin, Z., and Huang, X. (2012) Recent development in carbohydrate based anti-cancer vaccines. *J. Carbohydr. Chem. 31*, 143−186.
(24) Liptak, A., Jodal, I., Harangi, J., and Nanasi, P. (1983) Hydrogenolysis of the benzylidene acetals of thioglycosides with the Lialh4-Alcl3 reagent—synthesis of partially benzylated thioglycoside derivatives. *Acta Chim. Hung. 113*, 415−422.
(25) Ellervik, U., Grundberg, H., and Magnusson, G. (1998) Synthesis of lactam and acetamido analogues of sialyl Lewis X tetrasaccharide and Lewis X trisaccharide. *J. Org. Chem. 63*, 9323−9338.
(26) de Jong, A. R., Hagen, B., van der Ark, V., Overkleeft, H. S., Codee, J. D. C., and van der Marel, G. A. (2012) Exploring and exploiting the reactivity of glucuronic acid donors. *J. Org. Chem. 77*, 108−125.
(27) Wang, C. C., Kulkarni, S. S., Lee, J. C., Luo, S. Y., and Hung, S. C. (2008) Regioselective one-pot protection of glucose. *Nat. Protoc. 3*, 97−113.
(28) Huang, X., Huang, L., Wang, H., and Ye, X. S. (2004) Iterative one-pot synthesis of oligosaccharides. *Angew. Chem., Int. Ed. 43*, 5221−5224.
(29) Wang, Z., Zhou, L., El-Boubbou, K., Ye, X. S., and Huang, X. (2007) Multi-component one-pot synthesis of the tumor-associated carbohydrate antigen Globo-H based on preactivation of thioglycosyl donors. *J. Org. Chem. 72*, 6409−6420.
(30) Wang, Q., Ekanayaka, S. A., Wu, J., Zhang, J., and Guo, Z. (2008) Synthetic and immunological studies of 5′-*N*-phenylacetyl sTn to develop carbohydrate-based cancer vaccines and to explore the impacts of linkage between carbohydrate antigens and carrier proteins. *Bioconjugate Chem. 19*, 2060−2068.
(31) Buskas, T., Li, Y., and Boons, G. J. (2004) The immunogenicity of the tumor-associated antigen Lewis^{y} may be suppressed by a bifunctional cross-linker required for coupling to a carrier protein. *Chem.—Eur. J. 10*, 3517−3524.
(32) Wrolstad, R. E., Acree, T. E., and Decker, E. A. (2001) *Current Protocols in Food Analytical Chemistry*, John Wiley & Sons, Inc., New York.
(33) Jennings, H. J., and Sood, R. K. (1994) Synthetic glycoconjugates as human vaccines, in *Neoglycoconjugates: Preparation and Applications*, Academic Press, San Diego, CA.
(34) Markham, R. B., Pier, G. B., and Schreiber, J. R. (1991) The role of cytophilic IgG3 antibody in T-cell-mediated resistance to infection with the extracellular bacterium, *Pseudomonas aeruginosa*. *J. Immunol. 146*, 316−320.
(35) Gavin, A. L., Barnes, N., Dijstelbloem, H. M., and Hogarth, P. M. (1998) Cutting edge: Identification of the mouse IgG3 receptor: implications for antibody effector function at the interface between innate and adaptive immunity. *J. Immunol. 160*, 20−23.
(36) Casadevall, A. (1995) Antibody immunity and invasive fungal infections. *Infect. Immun. 63*, 4211−4218.
(37) Yuan, R. R., Casadevall, A., Spira, G., and Scharff, M. D. (1995) Isotype switching from IgG3 to IgG1 converts a nonprotective murine antibody to *Cryptococcus neoformans* into a protective antibody. *J. Immunol. 154*, 1810−1816.
(38) Mawas, F., Niggemann, J., Jones, C., Corbel, M. J., Kamerling, J. P., and Vliegenthart, J. F. (2002) Immunogenicity in a mouse model of a conjugate vaccine made with a synthetic single repeating unit of type 14 pneumococcal polysaccharide coupled to CRM197. *Infect. Immun. 70*, 5107−5114.
(39) Pozsgay, V., Chu, C., Pannell, L., Wolfe, J., Robbins, J. B., and Schneerson, R. (1999) Protein conjugates of synthetic saccharides elicit higher levels of serum IgG lipopolysaccharide antibodies in mice than do those of the O-specific polysaccharide from *Shigella dysenteriae* type 1. *Proc. Natl. Acad. Sci. U.S.A. 96*, 5194−5197.
(40) Kim, J., and Sudbery, P. (2011) *Candida albicans*, a major human fungal pathogen. *J. Microbiol. 49*, 171−177.

DOI: 10.1021/bc500575a
Bioconjugate Chem. 2015, 26, 466−476