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Synthesis of Glycoconjugate Vaccines for *Candida albicans* Using Novel Linker Methodology

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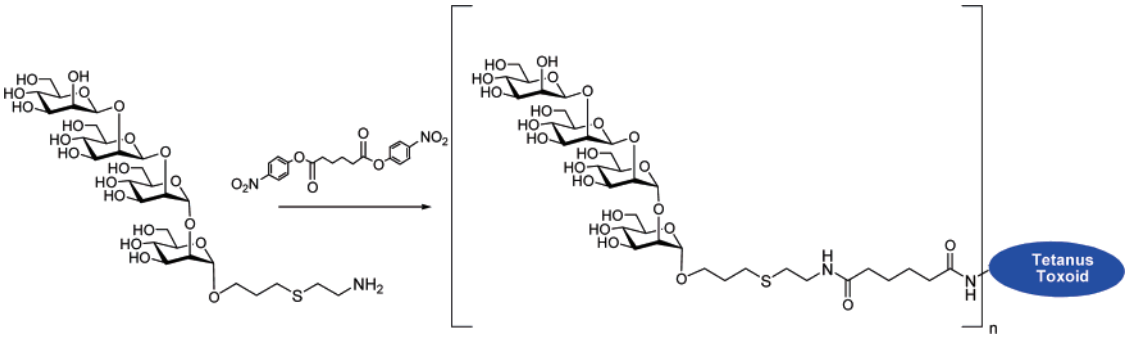

The cell wall phosphomannan of *Candida* species is a complex *N*-linked glycoprotein with a glycan chain that contains predominantly α-linked mannose residues. However, it is the minor *β*-mannan component of the phosphomannan of clinically important *Candida* strains that provides immunological protection in animal models of fungal disease and hence holds promise as a component of conjugate vaccines. This important antigen occurs in different forms linked to the α-mannan backbone via a phosphodiester bond (acid-labile *β*-mannan) or directly via a glycosidic bond. To reproducibly synthesize and evaluate conjugate vaccines, a robust method for the synthesis of the different oligosaccharide epitopes is required. Here, we report the gram-scale syntheses of both types of epitopes by an approach that utilizes glucosyl trichloroacetimidate donor **2** to first create a *β*-glucopyranoside linkage and then epimerizes the C-2 center via an oxidation−reduction sequence that provides an efficient multigram scale route to the *β*-mannopyranosides **5**, **8**, and **15**. Reaction of glycosides **16**−**18** with homobifunctional adipic acid *p*-nitrophenyl diesters in dry DMF gave the corresponding half esters in good yields, and of sufficient stability to permit chromatographic purification. Subsequent conjugation with BSA and tetanus toxiod (TT) under mild conjugation conditions afforded the corresponding tri- and tetrasaccharide neoglycoproteins with good efficiency. The conjugation method is also applicable to the coupling of small amounts (mg) of larger oligosaccharides with different proteins.

Introduction

Candida. albicans, the most common etiologic agent in candidiasis,[1] commonly affects immunocompromised patients and those undergoing long-term antibiotic treatment.[2] High mortality rates associated with *Candida*[3−5] and the increasing resistance of *C. albicans* to available antifungal drugs coupled with significant toxicity issues[6] have encouraged the consideration of vaccination strategies to increase host resistance to this pathogen.[7]

The cell wall phosphomannan antigen has received the greatest attention as it is highly immunogenic.[1] The glycan chain of this complex *N*-linked glycoprotein is composed of an extended (1→6)-α-D-mannopyranan backbone containing (1→2)-α-D-mannopyranan branches attached to which are shorter (1→2)-*β*-mannopyranan

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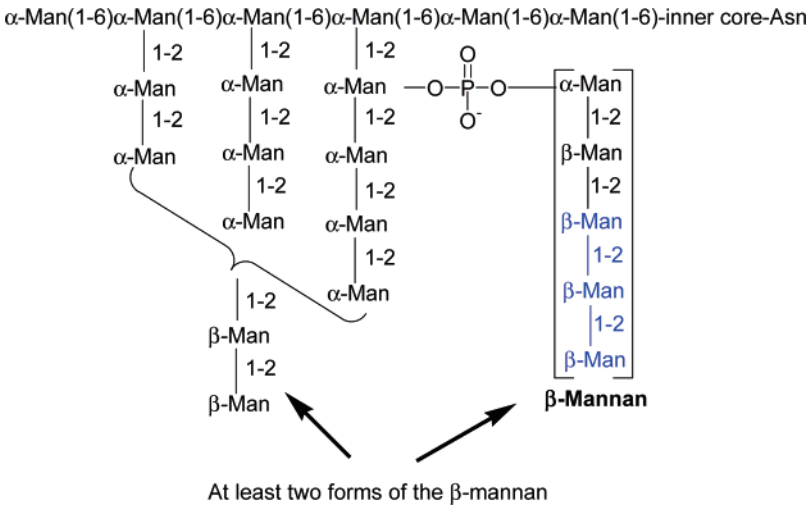

FIGURE 1. Structure of the cell wall phosphomannan antigen.

oligomers (Figure 1).[8−10] Both acid-labile and acid-stable β-mannans are present in *C. albicans* and can function as protective antigens. Attachment of the acid labile β−mannan occurs via a phosphodiester, but the exact attachment point has yet to be determined. Some of the (1→2)-β-mannan oligomers are linked directly to the α-mannan via a glycosidic bond and not via a phosphodiester. *C. albicans* serotype B is defined by the acid-labile β-mannan, while strains of serotype A have both acid-stable and acid-labile β-mannan epitopes.[9,10]

Compelling data has shown that the β-mannan portion of the *C. albicans* cell wall plays a decisive factor in pathogenicity, and further studies indicated that the active epitope is the (1→2)-β-mannan oligomer found in the phosphomannan.[11] Evidence from immunochemistry and solution properties of this antigen implied that (1→2)-β-mannan oligomers have potential as the key epitope of conjugate vaccines.[12] However, the precise oligosaccharide epitope which when conjugated to protein would provide the most effective vaccine remains a matter of conjecture. Immunochemical studies have shown that β-mannan epitopes are presented in the form of at least three distinct structural motifs and that more than one of these can be correlated with the antigenic factors 5 and 6 that characterize *C. albicans* serotypes A and B.[9,10] For serotype A and antigenic factor 6, Shibata and co-workers have proposed that the recognition size limit of the antibody to these antigens was four mannose units, Manβ1→2Manβ1→2Manα1→2Manα1→.[10] Evidence from our group indicated that protective IgM and IgG monoclonal antibodies that recognize antigenic factor 5 are most effectively inhibited by short oligosaccharide sequences such as di- and trisaccharide (1→2)-β-mannan oligomers.[13] These observations suggest that small synthetic oligomers representing these closely related but distinct antigenic determinants attached to protein are highly desirable for immunogical protection studies. Here, we describe the synthesis of oligosaccharides associated with antigenic factors 5 and 6 and their conjugation to carrier proteins.

Efficient construction of (1→2)-β-mannopyranosides remains a challenging task, since despite several novel approaches,[14−16] a general solution to the synthesis of this class of molecule has been elusive. Employing 4,6-*O*-benzylidene-protected mannopyranosyl sulfoxides as a glycosyl donor, Crich[17] and co-workers have successfully synthesized a variety of β-mannopyranosyl oligomers including 1,2-linked β-mannopyranosyl oligomers. Since we wished to develop a synthesis that could be easily scaled to the multigram level, we wanted to avoid the mandatory requirements of a 4,6-*O*-benzylidene acetal protecting group and the very low temperature of the coupling reaction for β-glycoside formation via this sulfoxide methodology.[17] Our successful application of an ulosyl bromide donor and selective stereoselective reduction was especially well suited to the construction of (1→2)-β-mannan oligomers[18] on a relatively small-scale, but due mainly to the lability of the glycosyl bromide donor this approach is not well suited to larger scale work. Gram-scale synthesis of complex β-mannan oligomers for the preparation of neoglycoconjugate requires stable and readily prepared synthons. A reliable method based on the formation of a β-glucopyranosyl linkage with subsequent C-2 epimerzation via an oxidation−reduction sequence has been employed by several authors beginning with Kotchetkov et al.,[19] Ekborg et al.,[20] and later

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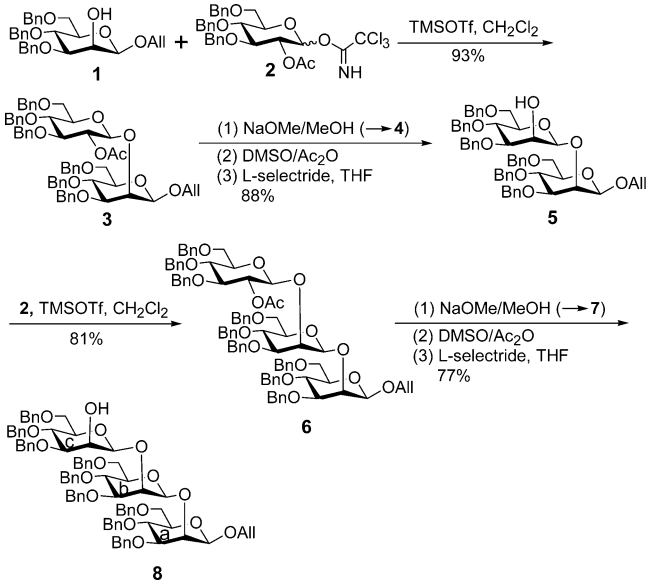
by other groups.[21] The group of Fraser-Reid recently reported an adaptation of this approach to the synthesis of (1→2)-β-linked oligomannnans of *C. albicans* via an ortho ester-based strategy.[22] Here, we report a related but simplified approach based on a trichloroacetimidate glucopyranosyl donor[23a,b] in combination with an oxidation−reduction strategy that constitutes a versatile method for the multigram-scale generation of (1→2)-β-mannan oligomers suitably functionalized for covalent attachment to immunogenic proteins.

The conjugation strategy whereby oligosaccharide is covalently linked to protein to yield a conjugate vaccine is a major factor that influences the synthetic strategy of oligosaccharide assembly and deprotection.[24] The chemistry of conjugation may further impart undesirable immunological properties to the vaccine. One of the most efficient coupling methods involves the use of the homobifunctional reagent, diethyl squarate,[25] which affords reproducible conjugation in high yields under mild conditions with small amounts of oligosaccharide and protein at low concentration. However, its use in conjugate vaccine application has been correlated with a reduced immune response to the oligosaccharide epitope[26] and with potential immune response to the squarate residue itself.[27] Recently, we have reported the preparation of neoglycoproteins employing the *p*-nitrophenyl ester of adipic acid as a homobifunctional coupling reagent that reacts efficiency with an amino terminated tether of an oligosaccharide under mild conditions.[28] Here, distinct trisaccharide and tetrasaccharide glycoconjugate vaccines against *C. albicans* were synthesized by this approach.

Result and Discussion

Synthesis of (1→2)-β-Linked Disaccharide and Trisaccharide. The synthesis of disaccharide and trisaccharide was accomplished as outlined in Scheme 1. The approach is based on a strategy that first establishes a β-D-glucopyranoside linkage and then epimerizes at C-2 via an oxidation−reduction sequence.[19−22] Building blocks **1**[18b] and **2**[29] are readily synthesized according to published literature. The glycosylation reaction between monosaccharide acceptor **1** and trichloroacetimidate glycosyl donor **2** was performed by activation with trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.05 equiv) in CH_2Cl_2 at −10 °C, affording the required disaccharide **3** in excellent yield. Deacetylation under Zemplën conditions gave the desired alcohol **4** in quantitative yield, and the resulting alcohol was oxidized to the corresponding keto derivative by DMSO and Ac_2O (2:1). Subsequent selective reduction with L-Selectride at −78 °C in THF afforded the target disaccharide **5** in 88% yield. After repetition of the glycosylation reaction with donor **2**, followed by a saponification, oxidation, and reduction sequence, trisaccharide **8** was obtained in 62% yield on a gram scale over four steps. Excellent diastereoselectivity was observed in this strategy since in the following reduction only trace amounts of the β-gluco epimer could be detected by ^{1}H NMR. This simplified the purification of the product, and most importantly, all the reactions could be performed on a multigram scale. Heteronuclear one-bond coupling constants ($^1J_{C-H}$) were used to unambiguously establish the anomeric configuration of the mannopyransyl residue.[30]

SCHEME 1. Synthesis of (1→2)-β-Linked Mannose Disaccharide and Trisaccharide



Synthesis of β-D-Man(1→2)-β-D-Man(1→2)-α-D-Man-(1→2)-α-D-Man Tetrasaccharide. Disaccharide **9** was synthesized according to a published procedure.[31] Reaction of disaccharide acceptor **9** with glycosyl donor **2** in CH_2Cl_2 at −10 °C in the presence of TMSOTf as catalyst (0.02 equiv) afforded the desired trisaccharide 10 in 90% yield. Trisaccharide **10** was transesterified with sodium methoxide in methanol to give alcohol **11**. Oxidation of trisaccharide **11** using acetic anhydride and DMSO, followed by reduction with L-Selectride at −78 °C in THF, gave trisaccharide **12** in good yield and high stereoselectivity (Scheme 2).

Glycosylation of trisaccharide **12** with donor **2** in the presence of TMSOTf (0.02 equivalents) in CH_2Cl_2 at −10 °C gave the required tetrasaccharide **13** in good yield. Subsequent deacetylation in a mixed solvent of CH_2Cl_2

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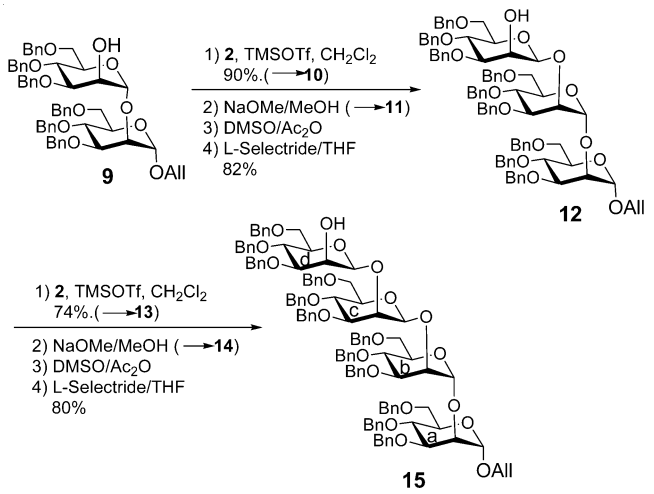
SCHEME 2. Synthesis of Tetrasaccharide 15

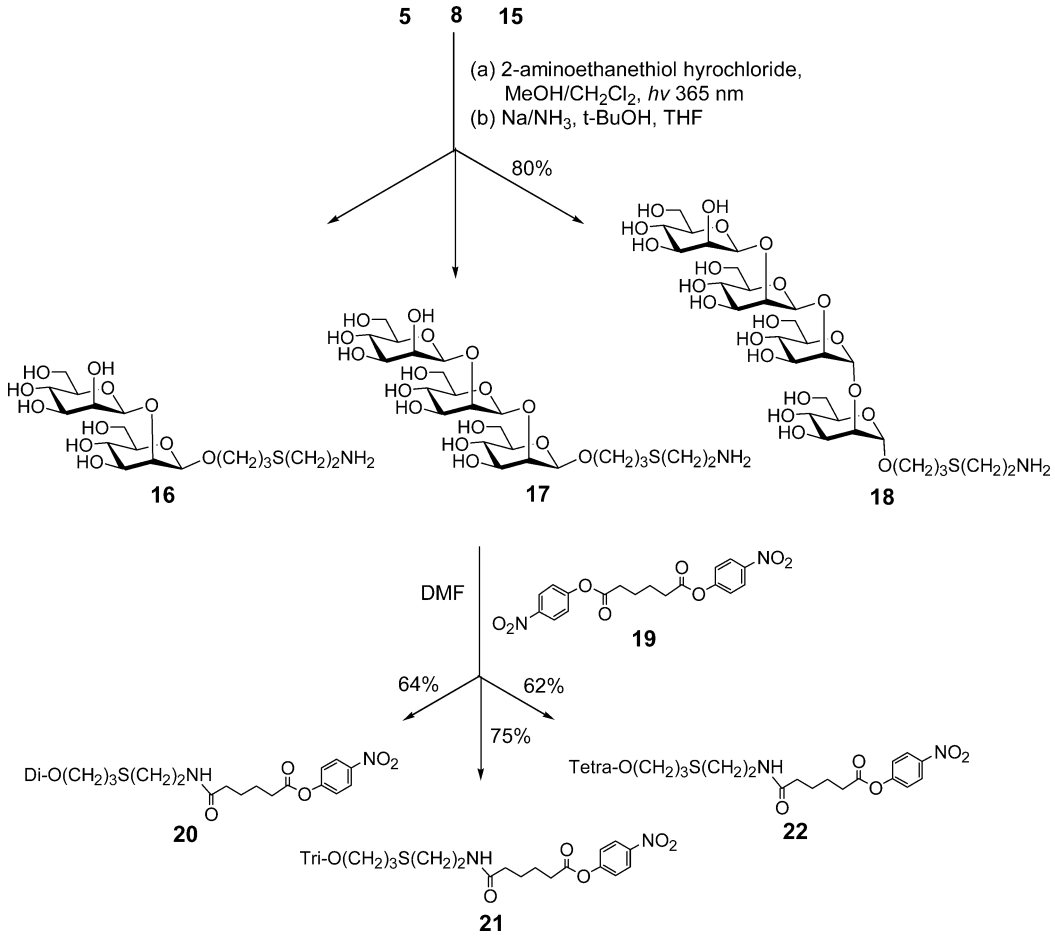


and MeOH (1:1) gave the β-glucopyranosyl alcohol **14**. Finally, oxidation and reduction as above afforded the desired tetrasaccharide **15** on a gram scale in 80% yield.

Synthesis of Half Esters 20−22. For the conjugation of deprotected oligosaccharide to protein, a terminal amine was chosen as a versatile functionality from which glycoconjugates could be readily generated.[18b] The protected oligosaccharides **5**, **8**, and **15** were elaborated via photoaddition of 2-aminoethanethiol[18b] to the allyl glycosides to give the amine-functionalized glycosides, and then subsequent deprotection under Birch conditions achieved the desired amino-functionalized glycosides **16**, **17**, and **18** in good yields. Previously, coupling of such compounds to bovine serum albumin (BSA) protein was achieved through a squarate linker.[18b,25a] Here, half esters of adipic acid phenyl ester were prepared according to our recently published procedure.[28] The oligosaccharide amines **16**, **17**, and **18** were treated with 5 equiv of linear homobifunctional *p*-nitrophenyl ester **19** in dry DMF at room temperature for 5 h, affording the corresponding half esters **20**, **21**, and **22** in good yield after purification on a reversed-phase column (Scheme 3). The reaction is readily monitored by TLC or UV spectroscopy, and the half esters are stable to both silica gel chromatography and reversed-phase isolation under acidic conditions. Excess linker could be easily removed by washing with dichloromethane and the yields of this reaction were in the range of 62−75%.

Formation of Neoglycoproteins. Coupling of half esters **20**, **21**, and **22** to BSA was performed by an 18 h incubation in buffer (pH = 7.5) at ambient temperature. The BSA conjugates **23**, **24**, and **25** were obtained as white powders after dialysis against deionized water followed by lyophilization (Scheme 4). In the same way, **21** and **22** were conjugated to tetanus toxoid (TT) in phosphate buffer (pH = 7.2) overnight at ambient temperature. After dialysis against phosphate buffered saline (PBS) pH = 7.2, the conjugates **26** and **27** were obtained for use as a vaccine. Targeted and observed incorpora-

SCHEME 3. Synthesis of Half Esters 20−22



SCHEME 4. Synthesis of Neoglycoproteins

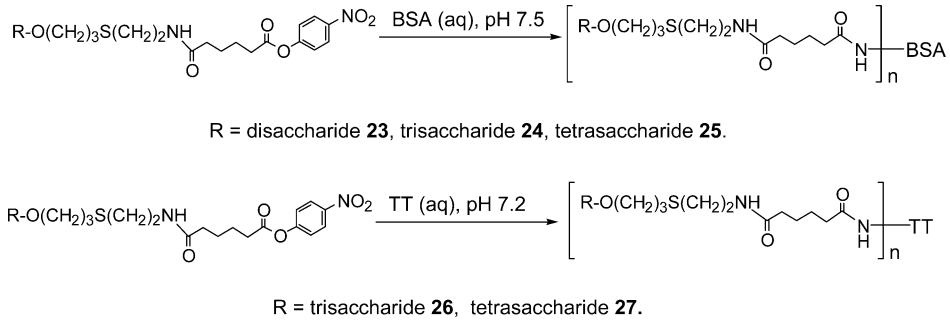


TABLE 1. BSA and TT Mannopyranan Conjugates

product	saccharide (mg)	molar ratio of protein/ monoester	hapten incorporated	incorporation efficiency (%)
23	1	1:30	13.4	45
24	1.6	1:20	8.8	44
25	2.3	1:30	11.5	38.3
26	3	1:40	13	32.5
27	2.6	1:30	12.6	41

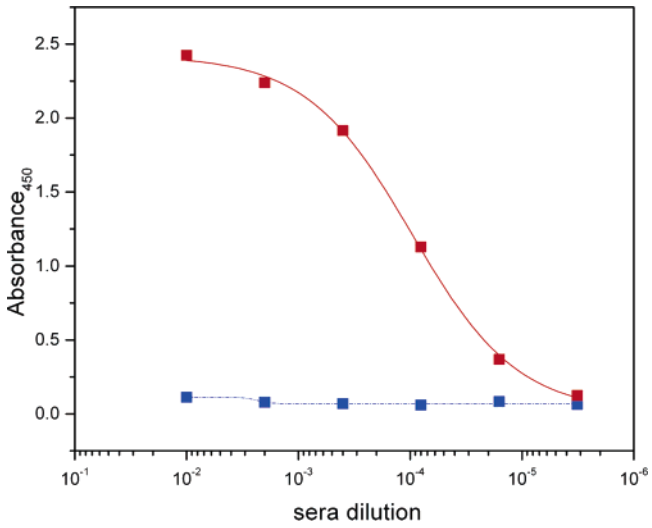


FIGURE 2. Typical titration curve for rabbit antisera raised against the trisaccharide tetanus toxoid conjugate **26**. Trisaccharide−BSA conjugate **24** was coated on ELISA plates, and serial diltions of sera were allowed to bind to the plate. The red curve represents antisera collected after two injections and the blue line the antibody titer of preimmune sera collected from the rabbit prior to the start of the experiment. The dilution of sera at which a significant absorbance reading is recorded (OD = 0.2) is recorded at the antibody titer.

tions are tabulated (Table 1). The degree of incorporation of the oligosaccharides on BSA or tetanus toxiod was established by MALDI-TOF MS using sinapinic acid as the matrix, and conjugation efficiencies of between 32.5 and 45% were achieved, similar to those published for the coupling of oligosaccharides to BSA.[28] This corresponds to the incorporation of 12 ligands to TT or BSA with a 30-fold molar excess of activated oligosaccharides.

Preliminary experiments established that rabbits immunized with either trisaccharide **26** or tetrasaccharide **27** succeeded in raising high titer trisaccharide specific antibodies that bound the corresponding BSA conjugates **24** and **25** but not unconjugated BSA. In the example

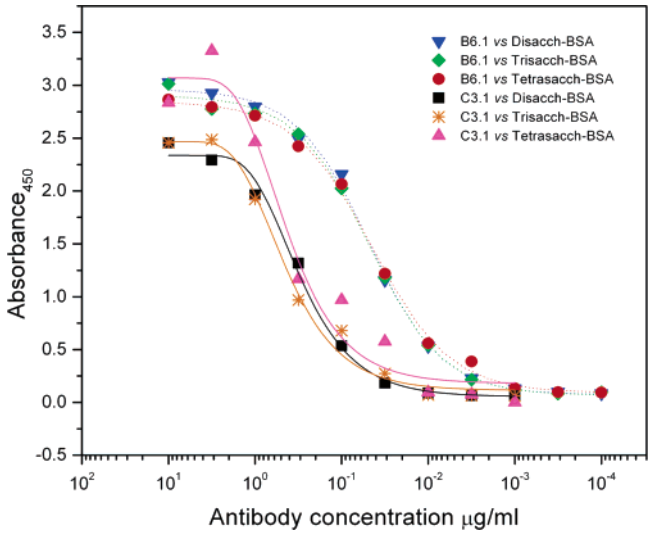


FIGURE 3. Protective monoclonal antibodies C3.1 and IgG3 and the IGM B6.1 previously described by Cutler et al.[11,12] were titrated against the 3 BSA conjugates **23**−**25**. The higher avidity decavalent IgM antibody titers to higher dilution than the divalent IgG antibody. The titration curves for each antibody with the three conjugates are seen to overlap.

presented here the immune response of rabbits to immunization with the trisaccharide antigen **26** results in reproducible antibody titers of ~1:100000 (Figure 2). These data will be reported as part of more detailed studies that document active protection of immunosuppressed rabbits to live challenge with otherwise lethal doses of *C. albicans*. These antibodies exhibited comparable titers when ELISA plates were coated with a crude *β*-mannan cell wall extract from *C. albicans*. When the three glycoconjugates **23**−**25** were used to coat ELISA plates and the two protective monoclonal antibodies IgG C3.1 and IgM B6.1 were titered against them, all three antigens strongly bound both antibodies. Furthermore, it can be seen that there was no preference for any of the three antigens even at high antibody dilutions where fine specificity effects might become evident. Disaccharide, trisaccharide, and tetrasaccharide conjugates **23**−**25** showed almost overlapping titration curves with each antibody (Figure 3). The curves show that each set of three titration curves cluster together, one set for the IgG monoclonal antibody C3.1 and the other for the IgM monoclonal antibody B6.1. The decavalency of the IgM antibody accounts for the shift of it titration curves toward higher antibody dilution. In agreement with our previously published inhibition data for homooligomers

from disaccharide through to hexasaccharide,[13,27] the near-identical binding curves strongly suggest that the binding sites of both antibodies are largely directed toward recognition of the common terminal disaccharide present in the three antigens **23**−**25**. We believe these results imply that a disaccharide or trisaccharide glycoconjugate would be an excellent candidate for a commercially viable synthetic conjugate vaccine that would be capable of eliciting a protective immune response for those individuals at risk from potentially life threatening *C. albicans* infections.

Conclusion

Distinct *C. albicans* (1→2)-*β*-D-mannopyranan epitopes have been synthesized on a multigram scale employing a glucopyranosyl imidate donor and C2 epimerization methodology. Coupling these oligosaccharides to BSA or tetanus toxoid using a linear homobifunctional linker was accomplished with high efficiency under mild conditions. Preliminary data show that the tetanus toxoid glycoconjugates **26** and **27** were very effective immunogens in rabbits and generated high titer saccharide and *C. albicans* specific antibodies. These data suggest that simple conjugate vaccines prepared from disaccharide or trisaccharide conjugated to immunogenic carrier protein such as tetanus toxoid may constitute an effective vaccine that is able to provide protection against *C. albicans* infections. The efficacy of these conjugates as protective *C. albicans* vaccines in rabbits and mice is the subject of continuing studies.

Experimental Section

Allyl (3,4,6-Tri-*O*-benzyl-2-*O*-acetyl-*β*-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranoside (3). Glycosyl donor **2** (1.52 g, 2.4 mmol), monosaccharide acceptor **1** (980 mg, 2 mmol), and activated 4 Å molecular sieves (200 mg) were dried together under vacuum for 1 h in a pear-shaped flask (50 mL). The contents of the flask were then dissolved in dichloromethane (10 mL). The suspension was stirred for 10 min at room temperature under argon, and then the temperature was reduced with a −10 °C bath, and trimethylsilyl trifluorometanesulfonate (18 *μ*L) was added dropwise. After 30 min, the reaction mixture was neutralized with triethylamine and concentrated in a vacuum. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate, 6:1) to afford **3** (1.79 g, 93%) as a white foam: $[\alpha]_D$ −31.1 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.18−7.42 (m, 30 H, Ar), 5.89 (m, 1H, $OCH_2CH{=}CH_2$), 5.32−5.37 (m, 1H, $OCH_2CH{=}CH_2$), 5.20−5.23 (m, 1H, $OCH_2CH{=}CH_2$), 5.13 (dd, ^3J = 8.0 Hz, 9.6 Hz, 1 H, 2b-*H*), 4.76−4.89 (m, 6 H, 1b-*H*, 5/2 CH_2Ph), 4.44−4.56 (m, 7 H, 7/2 CH_2Ph), 4.44−4.38 (m, 1 H, $OCH_2CH{=}CH_2$), 4.35 (s, 1 H, 1a-*H*), 4.28 (d, $J_{1,2}$ = 2.8 Hz, 1 H, 2a-*H*), 4.0−4.1 (m, 1 H, $OCH_2CH{=}CH_2$), 3.76−3.82 (m, 3 H, 3b-*H*, 6a-*H*, 6′a-*H*), 3.58−3.73 (m, 5 H, 4a-*H*, 4b-*H*, 5*b*-H, 6′b-*H*, 6b-*H*), 3.52 (dd, ^3J = 2.8 Hz, 9.2 Hz, 1 H, 3a-*H*), 3.46 (m, 1 H, 5a-*H*), 1.98 (s, 3 H, Ac); EMS calcd (M + Na) 987.4, found 987.4.

Allyl (3,4,6-Tri-*O*-benzyl-*β*-D-glucopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranoside (4). To a solution of **3** (2.55 g, 2.65 mmol) in methanol (20 mL) was added sodium methoxide (14 mg, 0.264 mmol) and the mixture stirred overnight at room temperature. The resulting mixture was neutralized with IR 120 (H^+ form), and concentrated in a vacuum. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate, 4:1) to afford **4** (2.44 g, 100%) as a white foam: $[\alpha]_D$ −39.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.22−7.44 (m, 30 H, Ar), 5.95 (m, 1H, $OCH_2CH{=}CH_2$), 5.32−5.37 (m, 1H, $OCH_2CH{=}CH_2$), 5.24−5.26 (m, 1H, $OCH_2CH{=}CH_2$), 5.09−5.11 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.83−4.89 (m, 4 H, 2 CH_2Ph), 4.75 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1b-*H*), 4.66 (d, ^2J = 12.0 Hz, 1H, 1/2 CH_2Ph), 4.52−4.59 (m, 6 H, 3 CH_2Ph), 4.46−4.48 (m, 2 H, 1a-*H*, $OCH_2CH{=}CH_2$), 4.31 (d, ^3J = 3.1 Hz, 1 H, 2a-*H*), 4.08−4.11 (m, 1 H, $OCH_2CH{=}CH_2$), 3.94 (t, ^3J = 9.5 Hz, 9.9 Hz, 1 H, 4a-*H*), 3.66−3.81 (m, 6 H, 2b-*H*, 3b-*H*, 6b-*H*, 6′b-*H*, 6a-*H*, 6′a-*H*), 3.54−3.62 (m, 3 H, 3a-*H*, 4b-*H*, 5b-*H*), 3.44 (m, 1 H, 5a-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 138.0−138.5, 133.5, 127.4−128.3, 117.7, 104.0 ($^1J_{C-H}$ = 162 Hz, C-1b), 99.3 ($^1J_{C-H}$ = 156 Hz, C-1a), 85.1, 80.3, 75.7, 75.4, 75.3, 75.0, 74.7, 74.5, 74.3, 73.4, 70.3, 69.9, 69.2; EMS calcd (M + Na) 945.3, found 945.4.

Allyl (3,4,6-Tri-*O*-benzyl-*β*-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranoside (5). Disaccharide **4** (1.5 g, 1.62 mmol) was dissolved in freshly distilled dimethyl sulfoxide (10 mL), and acetic anhydride (5 mL) was added. The resulting solution was stirred for 18 h at room temperature, diluted with ethyl acetate, and then washed with water, sodium bicarbonate solution, and a brine solution. Finally, the solution was concentrated at low pressure to give a yellow syrup. This syrup was dissolved in THF (20 mL) and then cooled to −78 °C under argon. L-Selectride (1 M THF, 6 mL) was added dropwise, and the reaction was stirred for 15 min. The dry ice bath was removed, and the reaction was allowed to warm to room temperature. The reaction mixture was quenched after 15 min with methanol (2 mL) and diluted with dichloromethane. Washing with a solution of hydrogen peroxide (5%) and sodium hydroxide (1 M) followed by sodium thiosulfate (5%) and sodium chloride solutions gave a clear colorless organic solution. The resulting solution was dried over magnesium sulfate and concentrated to a colorless oil. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate, 3:1) to afford **5** (1.32 g, 88%) as a white oil. The analytic data of compound **5** were identical with the published values.[18b]

Allyl (3,4,6-Tri-*O*-benzyl-2-*O*-acetyl-*β*-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-*β*-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranoside (6). The procedure used was analogous to the preparation of **3** and used glycosyl donor **2** (1.08 g, 1.68 mmol), disaccharide **5** (1.29 g, 1.41 mmol), dichloromethane (10 mL), trimethylsilyl trifluoromethanesulfonate (13 *μ*L), and activated 4 Å molecular sieves (200 mg). Column chromatography in *n*-hexane/ethyl acetate (4:1) gave the trisaccharide **6** (1.59 g, 81%): $[\alpha]_D$ −50.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.03−7.44 (m, 45 H, Ar), 5.86 (m, 1H, $OCH_2CH{=}CH_2$), 5.27 (d, $J_{1,2}$ = 8.4 Hz, 1 H, 1c-*H*), 5.17−5.20 (m, 2 H, 2c-*H*, $OCH_2CH{=}CH_2$), 5.08−5.11 (m, 1 H, $OCH_2CH{=}CH_2$), 4.94−4.98 (m, 3 H, 3/2 CH_2Ph), 4.80−4.86 (m, 2 H, CH_2Ph), 4.69−4.74 (m, 4 H, 1b-*H*, 3/2 CH_2Ph), 4.63 (d, ^2J = 13.2 Hz, 1 H, 1/2 CH_2Ph), 4.48−4.57 (m, 9 H, 2b-*H*, 4 CH_2Ph), 4.43 (d, ^2J = 12.0 Hz, 1 H, 1/2 CH_2Ph), 4.40 (s, 1 H, 1a-*H*), 4.35−4.38 (m, 1 H, $OCH_2CH{=}CH_2$), 4.20 (d, ^3J = 3.0 Hz, 1 H, 2a-*H*), 3.92 (t, ^3J = 8.4 Hz, 1 H, 3c-*H*), 3.65−3.81 (m, 8 H, 4c-*H*, 5b-*H*, 5c-*H*, 6c-*H*, 6a-*H*, 6′a-*H*, 6b-*H*, 6′b-*H*), 3.62 (t, ^3J = 9.6 Hz, 1 H, 4b-*H*), 3.47−3.55 (m, 3 H, 3a-*H*, 3b-*H*, 6′c-*H*), 3.37 (m, 1 H, 5a-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 138.6−138.2, 133.9, 128.4−127.4, 117.1, 102.4, 101.1, 100.2, 83.6, 80.6, 80.2, 78.3, 75.6, 75.5, 75.3, 75.2, 74.9, 74.7, 74.6, 74.5, 73.4, 73.3, 73.0, 72.0, 70.0, 69.8, 69.6; EMS calcd for $C_{86}H_{92}O_{17}Na$ 1419.6, found 1420.0.

Allyl (3,4,6-Tri-*O*-benzyl-*β*-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-*β*-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-*β*-D-mannopyranoside (7). The procedure used was analogous to the preparation of **4** and used trisaccharide **6** (1.59 g, 1.14 mmol), sodium methoxide (12 mg), dichloromethane (5 mL), and methanol (10 mL). Column chromatography in *n*-hexane/ethyl acetate (4:1) gave the trisaccharide **7** (1.54 g, 100%): $[\alpha]_D$ −54.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.04−7.46 (m, 45 H, Ar), 5.79−5.94 (m, 1H, $OCH_2CH{=}CH_2$), 5.23−5.26 (m, 1 H, $OCH_2CH{=}CH_2$), 5.18−5.20 (m, 1 H, $OCH_2CH{=}CH_2$), 5.02−5.07 (m, 5 H, 5/2 CH_2Ph),

4.98 (s, 1 H, 1b−H), 4.89 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.81 (d, $J_{1,2}$ =7.8 Hz, 1 H, 1c-*H*), 4.63−4.72 (m, 3 H, 3/2 CH_2-Ph), 4.39−4.56 (m, 13 H, 9/2 CH_2Ph, 2a-*H*, 2b-*H*, 1a-*H*, OCH_2-CH=CH_2), 4.15 (t, ^3J = 9.6 Hz, 1 H, 4b-*H*), 4.06 (m, 1 H, OCH_2CH=CH_2), 3.87 (t, ^3J = 9.6 Hz, 1 H, 4a-*H*), 3.66−3.84 (m, 8 H, 2c-*H*, 3c-*H*, 6a-*H*, 6′a-*H*, 6b-*H*, 6′b-*H*, 6c-*H*, 6′c-*H*), 3.52−3.63 (m, 5 H, 5c-*H*, 3a-*H*, 4c-*H*, 3b-*H*, 5a-*H*), 3.43 (m, 1 H, 5b-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 139.2−138.1, 133.8, 128.4−127.2, 117.4, 105.3, 100.1, 99.9, 86.7, 80.2, 80.1, 77.3, 75.6, 75.4, 74.9, 74.8, 74.7, 74.6, 74.1, 73.6, 73.4, 73.3, 71.1, 70.3, 70.2, 69.8; EMS calcd for $C_{84}H_{90}O_{16}Na$ 1377.6, found 1378.0.

Allyl (3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-β-D-mannopyranoside (8). The procedure used was analogous to the preparation of **5** and used trisaccharide **7** (1.07 g, 0.79 mmol), dimethyl sulfoxide (10 mL), acetic anhydride (5 mL), THF (10 mL), and L-Selectride (1 M, 3 mL). Column chromatography in *n*-hexane/ethyl acetate (5:2) gave the trisaccharide **8** (823 mg, 77%). The analytical data of compound **8** were identical with the published values.[18b]

Allyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (10). The procedure used was analogous to the preparation of **3** and used glycosyl donor **2** (1.52 g, 2.37 mmol), disaccharide **9** (1.83 g, 1.98 mmol), dichloromethane (10 mL), trimethylsilyl trifluoromethanesulfonate (7 μL), and activated 4 Å molecular sieves (200 mg). Column chromatography in *n*-hexane/ethyl acetate (6:1) gave the trisaccharide **10** (2.49 g, 90%): $[\alpha]_D$ +9.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.19−7.40 (m, 45 H, Ar), 5.79−5.85 (m, 1H, OCH_2CH=CH_2), 5.19−5.23 (m, 1 H, OCH_2CH=CH_2), 5.11−5.13 (m, 1 H, OCH_2CH=CH_2), 5.08 (dd, ^3J = 8.4 Hz, 9.6 Hz, 1 H, 2c-*H*), 5.01 (d, $J_{1,2}$ = 2.4 Hz, 1 H, 1a-*H*), 4.97 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1b-*H*), 4.91 (d, ^2J = 10.8 Hz, 1 H, 1/2 CH_2Ph), 4.66−4.87 (m, 8 H, 4 CH_2Ph), 4.47−4.62 (m, 8 H, 4 CH_2Ph), 4.42 (d, ^2J = 10.8 Hz, 1 H, 1/2 CH_2Ph), 4.28 (m, 1 H, 1c-*H*), 4.19 (t, ^3J = 5.4 Hz, 1 H, 2a-*H*), 4.12 (m, 1H, 2b-*H*), 4.08 (m, 1 H, OCH_2CH=CH_2), 3.98 (dd, ^3J = 3.0 Hz, 9.0 Hz, 1 H, 3b-*H*), 3.91−3.93 (m, 2 H, 3a-*H*, 6a-*H*), 3.77−3.85 (m, 4 H, 4b-*H*, OCH_2CH=CH_2, 6′a-*H*, 6b-*H*), 3.71−3.74 (m, 3 H, 4a-*H*, 5b-*H*, 6′c-*H*), 3.58−3.68 (m, 4 H, 6c-*H*, 6′b-*H*, 4c-*H*, 5a-*H*), 3.52 (t, ^3J = 9.0 Hz, 1 H, 3c-*H*), 3.88 (m, 1 H, 5c-*H*), 1.98 (s, 3 H, Ac); ^{13}C NMR (125 MHz, $CDCl_3$), 99.8, 99.4, 98.2, 82.6, 80.2, 77.9, 77.6, 75.1, 75.0, 74.8, 74.7, 74.4, 73.5, 73.4, 73.0, 72.8, 72.7, 71.9, 71.1, 70.3, 69.4, 67.9; ES HRMS calcd for $C_{86}H_{92}O_{17}Na$ 1419.623222, found 1419.623151.

Allyl (3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (11). Allyl trisaccharide (2.49 g) **10** was deacetylated using the protocol described above to give **11** (2.42 g, 100%): $[\alpha]_D$ +12.6 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.15−7.44 (m, 45 H, Ar), 5.79−5.85 (m, 1 H, OCH_2CH=CH_2), 5.19−5.22 (m, 1 H, OCH_2CH=CH_2), 5.11−5.13 (m, 2 H, 1a-*H*, OCH_2CH=CH_2), 4.95 (s, 1 H, 1b−H), 4.87 (m, 2 H, CH_2Ph), 4.77 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2-Ph), 4.44−4.73 (m, 14 H, 7 CH_2Ph), 4.35 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.23 (m, 1 H, 1c-*H*), 4.13 (m, 1 H, 2a-*H*), 4.09 (m, 1 H, OCH_2CH=CH_2), 4.04 (m, 1 H, 2b-*H*), 4.0 (dd, ^3J = 3.0 Hz, 6.6 Hz, 1 H, 3a-*H*), 3.74−3.93 (m, 8 H, 3b-*H*, 4a-*H*, 4b-*H*, OCH_2CH=CH_2, 5a-*H*, 6b-*H*, 6′b-*H*, 6a-*H*), 3.58−3.72 (m, 4 H, 5b-*H*, 6′a-*H*, 6c-*H*, 6′c-*H*), 3.42−3.52 (m, 3 H, 3c-*H*, 4c-*H*, 2c-*H*), 3.36 (m, 1 H, 5c-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 139.0−138.1, 133.9, 128.7−127.4, 117.0, 107.7, 100.5, 98.3, 84.2, 79.9, 77.5, 77.4, 77.3, 77.2, 77.0, 76.9, 76.8, 75.6, 75.3, 75.2, 75.1, 74.9, 74.7, 74.1, 73.5, 73.3, 73.2, 72.5, 72.4, 71.9, 69.6, 69.3, 67.9; ES HRMS calcd for $C_{84}H_{90}O_{16}Na$ 1377.612658, found 1377.612341.

Allyl (3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (12). Allyl trisaccharide (1.41 g) **11** was oxidized, and the keto derivative was reduced as outlined above to give **12** (1.16 g, 82%): $[\alpha]_D$ +1.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.11−7.39 (m, 45 H, Ar), 5.79−5.85 (m, 1 H, OCH_2CH=CH_2), 5.19−5.22 (m, 1 H, OCH_2CH=CH_2), 5.12−5.14 (m, 2 H, 1a-*H*, OCH_2CH=CH_2), 4.91−4.93 (m, 2 H, 1b−H, 1/2 CH_2Ph), 4.49−4.85 (m, 13 H, 13/2 CH_2Ph), 4.42 (m, 3 H, 2a-*H*, CH_2Ph), 4.34−4.36 (m, 3 H, 1c-*H*, CH_2Ph), 4.12 (m, 1 H, 2b-*H*), 4.07 (m, 1 H, OCH_2CH=CH_2), 4.02 (d, ^3J = 1.8 Hz, 1 H, 2c-*H*), 3.91−3.94 (m, 4 H, 3a-*H*, 3b-*H*, 6b-*H*, 6′b-*H*), 3.87 (t, ^3J = 9.0 Hz, 1 H, 4c-*H*), 3.83 (m, 1H, OCH_2CH=CH_2), 3.64−3.78 (m, 7 H, 4a-*H*, 4b-*H*, 5b-*H*, 5a-*H*, 6a-*H*, 6′a-*H*, 6c-*H*), 3.59 (m, 1 H, 6′c-*H*), 3.33 (d, ^3J = 9.0 Hz, 1 H, 3c-*H*), 3.19 (m, 1 H, 5c-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 138.7−138.1, 133.9, 128.5−127.4, 117.2, 99.6, 98.2, 97.3, 81.0, 80.3, 77.3, 75.2, 75.1, 75.0, 74.8, 74.7, 74.5, 74.3, 74.1, 73.4, 73.3, 73.2, 72.8, 72.0, 71.9, 71.6, 71.1, 70.8, 69.5, 69.3, 67.9, 67.8; ES HRMS calcd for $C_{84}H_{90}O_{16}Na$ 1377.612658, found 1377.612657.

Allyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (13). Allyl trisaccharide **12** (1.08 g) was glycosylated as outlined above to give **13** (1.08 g, 74%): $[\alpha]_D$ −10.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 7.04−7.40 (m, 60 H, Ar), 5.78−5.84 (m, 1 H, OCH_2CH=CH_2), 5.17−5.20 (m, 1 H, OCH_2CH=CH_2), 5.15 (t, ^3J = 9.0 Hz, 1 H, 2d-*H*), 5.06−5.10 (m, 2 H, 1d-*H*, OCH_2CH=CH_2), 4.99 (s, 1 H, 1a-*H*), 4.95 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.76−4.91 (m, 6 H, 1c-*H*, 1b-*H*, 2 CH_2Ph), 4.41−4.69 (m, 18 H, 9 CH_2Ph), 4.37 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.22 (br, 1 H, 2a-*H*), 4.18 (d, ^3J = 3.0 Hz, 1 H, 2b-*H*), 4.07 (m, 1 H, OCH_2CH=CH_2), 4.04 (m, 1 H, 2c-*H*), 3.77−3.95 (m, 6 H, OCH_2CH=CH_2, 3d-*H*, 5a-*H*, 3a-*H*, 3c-*H*, 6c-*H*), 3.55−3.75 (m, 13 H, 4a-*H*, 4b-*H*, 4c-*H*, 4d-*H*, 5c-*H*, 5d-*H*, 5b-*H*, 6a-*H*, 6′a-*H*, 6′c-*H*, 6d-*H*, 6b-*H*, 6′b-*H*), 3.26−3.31 (m, 2 H, 6′d-*H*, 3b-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 138.8−137.9, 133.8, 128.6−127.3, 117.2, 101.0, 99.5, 98.0, 83.5, 79.9, 78.2, 78.1, 76.8, 75.4, 75.2, 75.1, 75.0, 74.9, 74.8, 74.7, 74.5, 74.4, 73.5, 73.3, 73.2, 72.7, 72.3, 71.8, 71.7, 70.5, 69.6, 69.5, 69.3, 69.2, 69.1, 67.8; ES HRMS calcd for $C_{113}H_{120}O_{22}Na$ 1851.816897, found 1851.817052.

Allyl (3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (14). Allyl tetrasaccharide **13** (1.08 g) was deacetylated as outlined above to give **14** (1.05 g, 100%): $[\alpha]_D$ −26.7 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 6.93−7.42 (m, 60 H, Ar), 5.85−5.92 (m, 1 H, OCH_2CH=CH_2), 5.26−5.29 (m, 1 H, OCH_2CH=CH_2), 5.18−5.20 (m, 1 H, OCH_2CH=CH_2), 5.15 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1b-*H*), 5.07 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.86−5.02 (m, 7 H, 1a-*H*, 3 CH_2-Ph), 4.74−4.77 (m, 2 H, 2 CH_2Ph), 4.68 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1d-*H*), 4.67 (d, ^2J = 11.4 Hz, 1 H, 1/2 CH_2Ph), 4.53−4.64 (m, 8 H, 4 CH_2Ph), 4.31−4.48 (m, 5 H, 2b-*H*, 2 CH_2Ph), 4.14−4.20 (m, 3 H, 2c-*H*, 2a-*H*, 4b-*H*), 4.04 (s, 1 H, 1c-*H*), 3.87−3.97 (m, 5 H, 5b-*H*, 3a-*H*, 3b-*H*, 6d-*H*, 6′d-*H*), 3.78−3.82 (m, 4 H, 4c-*H*, 4a-*H*, 2d-*H*, 6′a-*H*), 3.63−3.74 (m, 7 H, 5a-*H*, 3d-*H*, 6b-*H*, 6′b-*H*, 6c-*H*, 6′c-*H*, 6′a-*H*), 3.59 (m, 1 H, 5d-*H*), 3.55 (t, ^3J = 8.4 Hz, 1 H, 4d-*H*), 3.28 (dd, ^3J = 3.6 Hz, 9.6 Hz, 1 H, 3c-*H*), 3.07 (m, 1 H, 5c-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 139.1−137.9, 133.7, 128.6−127.2, 117.4, 105.3 ($^1J_{C-H}$ = 165 Hz), 99.2 ($^1J_{C-H}$ = 170 Hz), 97.98 ($^1J_{C-H}$ = 170 Hz), 97.8 ($^1J_{C-H}$ = 160 Hz), 86.6, 80.4, 78.9, 77.9, 77.3, 76.9, 76.8, 75.3, 75.2, 75.1, 74.9, 74.8, 74.7, 74.6, 74.2, 74.0, 73.7, 73.4, 73.3, 73.2, 73.1, 71.9, 71.7, 70.6, 69.8, 69.7, 69.4, 69.3, 69.1, 67.8; ES HRMS calcd. for $C_{111}H_{118}O_{21}Na$ 1809.806332, found 1809.806374.

Allyl (3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (15). Allyl tetrasaccharide (600 mg) **14** was oxidized, and the keto derivative was reduced as outlined above to give **15** (480 mg, 80%): $[\alpha]_D$ −21.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ = 6.95−7.42 (m, 60 H, Ar), 5.82−5.89 (m, 1 H, OCH_2CH=CH_2), 5.82−5.89 (m, 1 H,

$OCH_2CH{=}CH_2$), 5.23−5.26 (m, 2 H, 1b−H, $OCH_2CH{=}CH_2$), 5.07 (s, 1 H, 1d-*H*), 5.02 (d, ^2J = 10.8 Hz, 1 H, 1/2 CH_2Ph), 4.82−4.91 (m, 6 H, 1a-*H*, 5/2 CH_2Ph), 4.42−4.76 (m, 19 H, 2b-*H*, 2c-*H*, 17/2 CH_2Ph), 4.35 (d, ^2J = 12.0 Hz, 1 H, 1/2 CH_2Ph), 4.12−4.27 (m, 6 H, 2d-*H*, 1c-*H*, 2a-*H*, 6a-*H*, 6′a-*H*, OCH_2-$CH{=}CH_2$), 3.84−4.01 (m, 6 H, 3a-*H*, 3b-*H*, 5b-*H*, 4d-*H*, 6d-*H*, $OCH_2CH{=}CH_2$), 3.82 (t, ^3J = 9.6 Hz, 1 H, 4a-*H*), 3.69−3.79 (m, 3 H, 6′d-*H*, 6b-*H*, 6′b-*H*), 3.63−3.69 (m, 4 H, 5a-*H*, 4b-*H*, 6c-*H*, 6′c-*H*), 3.51 (m, 1 H, 5d-*H*), 3.44 (dd, ^3J = 3.0 Hz, 9.0 Hz, 1 H, 3d-*H*), 3.27 (dd, ^3J = 3.6 Hz, 9.6 Hz, 1H, 3c-*H*), 2.99 (m, 1 H, 5c-*H*); ^{13}C NMR (125 MHz, $CDCl_3$), 139.1−137.8, 133.7, 128.9−127.1, 117.4, 99.9 ($^1J_{C-H}$ = 165 Hz), 98.4 ($^1J_{C-H}$ = 170 Hz), 98.0 ($^1J_{C-H}$ = 170.0 Hz), 97.7 ($^1J_{C-H}$ = 155 Hz), 83.1, 80.4, 79.3, 77.8, 77.3, 75.2, 75.1, 74.8, 74.7, 74.4, 74.3, 74.1, 73.6, 73.4, 73.2, 72.1, 71.5, 71.2, 70.2, 69.9, 69.6, 69.2, 68.7, 67.8, 67.3; ES HRMS calcd for $C_{111}H_{118}O_{21}Na$ 1809.806332, found 1809.806131.

3-(2-Aminoethylthio)propyl (β-D-Mannopyranosyl)-(1→2)-(β-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (18). Compound **15** (80 mg, 0.044 mmol) was reacted with 2-aminoethanethiol (250 mg, 2.2 mmol) under UV conditions and subsequent debenzylation under Birch conditions as reported in the literature[18b] to give free amine **18** (27.5 mg, 80%): ^{1}H NMR (600 MHz, D_2O) δ = 5.14 (m, 1 H, 1b-*H*), 5.07 (s, 1 H, 1a-*H*), 4.87 (s, 1 H, 1c-*H*), 4.85 (s, 1 H, 1d-*H*), 4.27 (m, 2 H, 2b-*H*, 2c-*H*), 4.15 (d, ^3J = 3.0 Hz, 1 H, 2d-*H*), 3.97 (m, 1 H, 2a-*H*), 3.54−3.93 (m, 20 H, 3a-*H*, 3b-*H,* 3c-*H*, 3d-*H*, 4a-*H*, 4b-*H*, 4c-*H*, 4d-*H*, 5a-*H*, 5b-*H*, 6a-*H*, 6′a-*H*, 6b-*H*, 6′b-*H*, 6c-*H*, 6′c-*H*, 6d-*H*, 6′d-*H*, OCH_2CH_2), 3.35−3.39 (m, 2 H, 5c-*H*, 5d-*H*), 3.21 (t, ^3J = 7.2 Hz, 1H, CH_2-NH_2), 2.66−2.68 (m, 4 H, $SCH_2CH_2NH_2$, $OCH_2CH_2CH_2S$), 1.92 (m, 2 H, $OCH_2CH_2CH_2S$); ^{13}C NMR (125 MHz, D_2O), 101.8 ($^1J_{C-H}$ = 162 Hz, C-1d), 101.1 ($^1J_{C-H}$ = 174 Hz, C-1b), 99.8 ($^1J_{C-H}$ = 156 Hz, C-1c), 99.1 ($^1J_{C-H}$ =168 Hz, C-1a), 79.6, 79.4, 78.6, 77.2, 74.2, 73.8, 73.7, 73.0, 71.3, 71.1, 70.2, 68.1, 67.8, 67.6, 67.1, 62.0, 61.8, 61.6, 61.5; EMS calcd for $C_{29}H_{53}NO_{21}$-SH^+ 784.3, found 784.4.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl (β-D-Mannopyranosyl)-(1→2)-β-D-mannopyranoside (20). To a solution of free amine **16** (10 mg, 0.022 mmol) in dry DMF (1 mL) was added diester **19** (42 mg, 0.11 mmol) under argon, and the mixture was stirred for 5.0 h when TLC indicated almost complete reaction of free amine. Finally, the reaction mixture was coevaporated with toluene to remove DMF, and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with H_2O (10 mL) containing 1% acetic acid. The water solution was then passed through a C18-Sep-Pac cartridge and eluted with methanol containing 1% acetic acid to remove any compound that would be irreversibly absorbed to the reversed-phase silica column. The solution was concentrated at low pressure to afford crude product as a solid. Final purification on reversed-phase silica (C18) was accomplished with a water/methanol mixture containing 1% acetic acid gradient to yield pure half ester **20** (9.8 mg, 64%): ^{1}H NMR (600 MHz, CD_3-OD) δ = 8.28 (m, 2 H, C_6H_2), 7.38 (m, 2 H, C_6H_2), 4.78 (s, 1 H, 1b-*H*), 4.56 (s, 1 H, 1a-*H*), 4.11 (d, ^3J = 3.0 Hz, 1 H, 2a-*H*), 3.98 (m, 2 H, 2b-*H*, OCH_2CH_2), 3.86 (m, 2 H, 6a-*H*, 6b-*H*), 3.60−3.72 (m, 3 H, $OCH_2CH_2CH_2$, 6′a-*H*, 6′b-*H*), 3.49−3.54 (m, 2 H, 4a-*H*, 4b-*H*), 3.44−3.46 (dd, ^3J = 3.5 Hz, 9.5 Hz, 1 H, 3a-*H*), 3.39−3.42 (dd, ^3J = 3.5 Hz, 9.5 Hz, 1 H, 3b-*H*), 3.37 (t, ^3J = 3.5 Hz, 2 H, CH_2COO), 3.17−3.21 (m, 2 H, 5a-*H*, 5b-*H*), 2.60−2.69 (m, 6 H, $NHCOCH_2$, CH_2CH_2NH, $COCH_2CH_2$), 2.26 (t, 2 H, SCH_2CH_2NH), 1.86 (m, 2 H, $OCH_2CH_2CH_2S$), 1.75 (m, 4 H, $CH_2CH_2CH_2CH_2$); EMS calcd for $C_{29}H_{44}N_2O_{16}SNa$ 731.24, found 731.2.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl (β-D-Mannopyranosyl)-(1→2)-(β-D-mannopyranosyl)-(1→2)-β-D-mannopyranoside (21). Free amine **17** (12.5 mg, 0.02 mmol) was reacted with diester **19** (40 mg, 0.1 mmol) as above outlined to give the half ester **21** (13 mg, 75%): ^{1}H NMR (600 MHz, CD_3OD) δ = 8.28 (m, 2 H, C_6H_2), 7.38 (m, 2 H, C_6H_2), 4.94 (s, 1 H, 1c-*H*), 4.78 (s, 1 H, 1b-*H*), 4.56 (s, 1 H, 1a-*H*), 4.22 (d, ^3J = 3.0 Hz, 1 H, 2b-*H*), 4.04 (m, 2 H, 2a-*H*, 2c-*H*), 4.0 (m, 1 H, OCH_2CH_2), 3.97−3.99 (m, 3 H, 6a-*H*, 6b-*H*, 6c-*H*), 3.62−3.88 (m, 4 H, 6′a-*H*, 6′b-*H*, 6′c-*H*, OCH_2CH_2), 3.41−3.55 (m, 6 H, 3a-*H*, 3b-*H*, 3c-*H*, 4a-*H*, 4b-*H*, 4c-*H*), 3.34−3.38 (m, 2 H, CH_2COO), 3.17−3.31 (m, 3 H, 5a-*H*, 5b-*H*, 5c-*H*), 2.62−2.67 (m, 6 H, $NHCOCH_2$, CH_2CH_2NH, $COCH_2CH_2$), 2.26 (t, 2 H, SCH_2CH_2NH), 1.87−1.97 (m, 2 H, OCH_2CH_2-CH_2S), 1.72−1.78 (m, 4 H, $CH_2CH_2CH_2CH_2$); EMS calcd for $C_{35}H_{54}N_2O_{21}SNa$ 893.29, found 893.3.

7-Aza-8,13-dioxo-13-(4-nitrophenoxy)-4-thiatridecanyl (β-D-Mannopyranosyl)-(1→2)-(β-D-mannopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→2)-α-D-mannopyranoside (22). Free amine **18** (9 mg, 0.01 mmol) was reacted with diester **19** (22 mg, 0.05 mmol) as above outlined to give the half ester **22** (7 mg, 62%): ^{1}H NMR (600 MHz, CD_3OD) δ = 8.31 (m, 2 H, C_6H_2), 7.38 (m, 2 H, C_6H_2), 5.05 (d, 1 H 1b-*H*), 5.02 (d, 1 H, 1a-*H*), 4.82 (s, 1 H, 1c-*H*), 4.73 (s, 1 H, 1d-*H*), 4.16 (m, 1 H, 2b-*H*), 4.12 (d, ^3J = 3.0 Hz, 1 H, 2d-*H*), 4.04 (d, ^3J = 3.0 Hz, 1 H, 2c-*H*), 3.40−3.88 (m, 21 H, 2a-*H*, 3a-*H*, 4a-*H*, 5a-*H*, 6a-*H*, 6′a-*H*, 3b-*H*, 4b-*H*, 6b-*H*, 6′b-*H*, 3c-*H*, 4c-*H*, 5c-*H*, 6c-*H*, 6′c-*H*, 3d-*H*, 4d-*H*, 6d-*H*, 6′d-*H*, $OCH_2CH_2CH_2S$), 3.36 (t, 2 H, CH_2NHCO), 3.23 (m, 2 H, 5b-*H*, 5d-*H*), 2.64−2.67 (m, 6 H, CH_2CH_2S, SCH_2CH_2NH, $NHCOCH_2$), 2.28 (t, 2 H, CH_2-COO), 1.73−1.86 (m, 6 H, $OCH_2CH_2CH_2S$, $OCCH_2CH_2CH_2$-CH_2CO); EMS calcd for $C_{41}H_{64}N_2O_{26}SNa$ 1055.2, found 1055.3.

Glycoconjugates. The general procedure for generating protein−carbohydrate conjugates was as followed: BSA (10 mg) was dissolved in phosphate buffer pH 7.5 (2 mL), the half ester was dissolved in DMF (100 μL), and then the solution was injected into the reaction medium slowly and the reaction was left for 1 day at room temperature. The mixture was then diluted with deionized water and dialyzed against 5 changes of deionized water (2 L) or started as a PBS solution pH = 7.2 for tetanus toxoid (TT) conjugates. The solution was lyophilized to a white solid.

MALDI-MS (positive mode, matrix sinapinic acid, H_2O): disaccharide−BSA conjugate **23** (74092); trisaccharide−BSA conjugate **24** (72823); tetrasaccharide−BSA conjugate **25** (76685); trisaccharide−TT conjugate **26** (159555); tetrasaccharide−TT conjugate **27** (160907).

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Supporting Information Available: ^{1}H spectra data for all new compounds and MAIDI-TOF data for glycoconjugates **23**−**27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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