Biotin Sulfone as a New Tool for Synthetic Oligosaccharide Immobilization: Application to Multiple Analysis Profiling and Surface Plasmonic Analysis of Anti-*Candida albicans* Antibody Reactivity against α and β (1→2) Oligomannosides†

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As a part of our glycoantigen synthetic program for diagnosis and basic analysis of yeast-related pathogenic mechanisms, a library of 1→2 oligomannosides suitable for immunoanalysis was prepared. The use of biotin sulfone, an oxidized form of biotin, offers a convenient solution for both oligosaccharide synthesis and immobilization on microspheres and surface plasmon resonance sensors. The application of this new strategy for the analysis of anti-*Candida albicans* antibody response through multiple-analyte profiling technology (Luminex) and with surface plasmonic analysis using biotin tagged synthetic oligosaccharides on avidin coated surfaces was validated using monoclonal antibodies.

1. Introduction

Oligosaccharides are important bacterial and fungal antigens. Being conjugated to proteins or lipids, oligosaccharides elicit strong antibody responses as a result of infections or immunological disorders.[1−8] Antioligosaccharide antibodies are widely used as biomarkers of these different human pathological conditions, whereas polyclonal or monoclonal antioligosaccharide antibodies raised in animals are used to decipher microbial biosynthetic and expression pathways in relation to pathogenesis.[9,10] Both basic and clinical applications are based on the assessment of antioligosaccharide antibody specificity, which is conferred by the nature of constitutive sugar units, the type of linkage, and the length of the oligosaccharide chain.[11] The antigenic oligosaccharidic fragments used in these assays are usually extracted from microbial cell walls by moderate degradation of polysaccharides or glycoproteins. These hydrophilic and water-soluble mixtures of antigens need to be immobilized to a surface, which is not an easy task. This problem is nicely solved with synthetic oligosaccharides; these can be prepared in a pure form and with a handle bearing an anchoring group. Probably the more convenient and universal of these anchoring groups is biotin. It allows a very strong and selective binding to a large number of surfaces: arrays, microtiter plates, microspheres. The binding to surfaces through avidin allows a precise coating with a controlled density. Biotin can be added to an unprotected synthetic oligosaccharide, but this solution does not show a distinct advantage over the use of natural saccharide. The coupling reaction between a hydrophilic oligosaccharide and hydrophobic biotin is not straightforward. It is preferable to add the lipophilic biotin at an earlier stage of synthesis than it is to couple it to a lipophilic protected sugar in an organic solvent compatible with both reactants. Unfortunately, the sulfur present in biotin interferes with a large number of reactions, in particular with the widely used hydrogenolysis of the benzyl group.

Biotin sulfone was first isolated as a natural metabolite of biotin.[12] Similar to biotin, it is strongly recognized by avidin (binding ratio to avidin vs biotin: 0.33).[13] Biotin sulfone has a reduced nucleophilicity and was expected to be compatible with benzyl hydrogenolysis. It is readily prepared from biotin by peracid oxidation.[14] Solange Lavielle's group used this tag, easily deuterated due to the presence of a sulfone group, for mass spectroscopy identification of tagged molecules.[15] However, it was never used for immunoassays.

The surface of the pathogenic yeast *Candida albicans* is covered by a phosphopepdidomannan (usually designated as mannan), which is composed of a complex repertoire of oligomannose sequences[11] that vary according to the growth conditions including *in vivo* in the host.[16−18] Immunochemical studies on mannan-derived oligomannosides as well as pioneering studies involving synthetic oligomannosides have established that members of this oligomannoside repertoire act as specific adhesins and/or epitopes.[19] Among these epitopes some detect antibodies associated with inflammatory disorders,[20] some support a protective antibody response,[21] and others do not.[22] In this context, it is essential for both basic research and diagnosis to dispose of a convenient and versatile tool for large scale analysis of the nature of the antibody response against individual oligomannoses in relation to yeast triggered pathogenic mechanisms.

For the above reasons, we envisaged that oligosaccharide construction from a biotin sulfone could be adapted to such an objective.

† Dedicated to Professor Marc Julia on the occasion of his 85th birthday.

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[a] Abbreviations: BSTO, biotin sulfone tagged oligosaccharides; EDC, (1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride; NIS, *N*-iodosuccinimide; SPR, surface plasmon resonance; TfOH, triflic acid.

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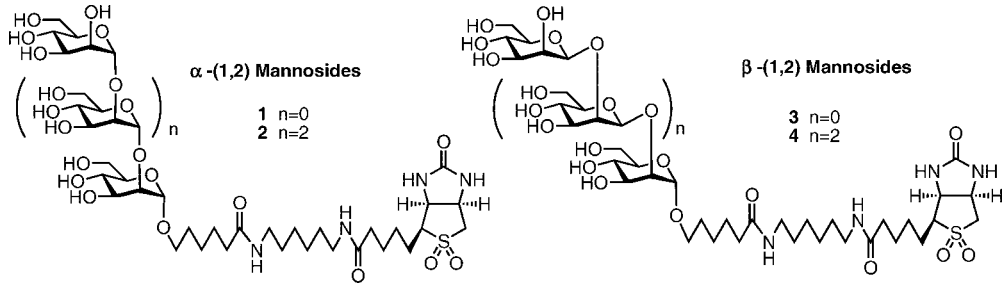
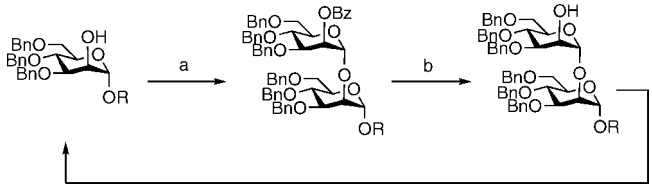


Figure 1. Synthesized biotin sulfone tagged oligosaccharides (BSTO).

Scheme 1. Iterative Synthesis of α (1→2) Oligomannosides Repeated until the Tetrasaccharide **20** is Obtained[a]



[a] Reagents and conditions: (a) **5**, NIS (*N*-iodosuccinimide), TfOH, 4 Å molecular sieves, CH_2Cl_2, rt; (b) MeONa, MeOH.

We therefore constructed a small library of biotin sulfone tagged oligosaccharides (BSTO[a]) (Figure 1) with the aim of assessing the feasibility and specificity of this strategy in differentiating anti-*C. albicans* β 1→2 oligomannoses, which are protective in a systemic model of candidiasis, and anti-α 1→2 oligomannoses, which are not. As a reference, we used monoclonal antibodies whose specificity against these oligomannose sequences has been established previously. Considering present technological evolution in immunoanalysis, the assays were performed using either multiple analyte profiling technology (Luminex) or surface plasmon resonance analysis (BIAcore). The combination of both methods, which required the coupling of BSTOs to fluorescent microspheres and SPR surfaces, was intended to explore the versatility of the same probes for implementing snapshots and kinetic analysis of antibody reactivities.

2. Glycoconjugate Synthesis

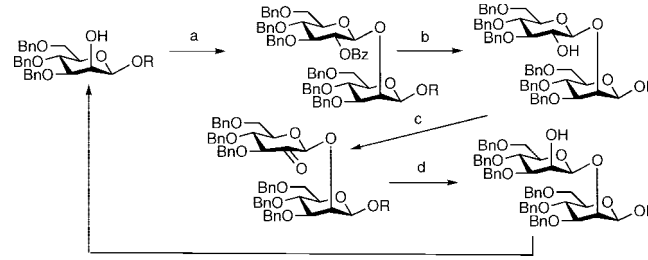
Synthesis of the Oligosaccharide Part. Preparation of the glycoconjugates **1**−**4** is divided into two stages. The first involves the synthesis of protected precursors **16**, **20**, **23**, and **29**, while the second involves the introduction of biotin followed by final deprotection, to give, respectively, compounds **1**, **2**, **3**, **4**. Synthesis of the precursors is shown in Schemes 1 and 2 and relies on two elongating blocks **5** and **6** (Scheme 3). Compound **6** is a thionaphth-2-yl mannoside bearing a participating group (benzoate) in position two. It is a convenient mannosyl donor for α (1→2) mannoside iterative synthesis through a two-step sequence: glycosylation−saponification (Schemes 1, 4).

Compound **6** is the analogous compound in the β-gluco series. It allows the construction of β-mannosides in a four step sequence: glycosylation−saponification−oxidation−reduction (Schemes 2, 5).

Although recent advances have been made in β (1→2)-mannoside synthesis by the Crich,[23] Bundle,[24] and Fraser Reid[25] groups since our first synthesis published in 1994,[26a,b] this method[27] is reliable and well adapted for 1→2 mannoside preparation. The reduction step provides a free alcohol ready for glycosylation. The hydride reduction occurs from the less hindered α face of the ketone to give the *manno* isomer. It was also very convenient for large scale preparation. In particular, this procedure avoids sensitive and expensive reagents and the usually difficult separation of an α/β mixture: indeed, for both linkages, the stereoselectivity relies on a participating group in position 2. The glycosyl donors **5** and **6** were prepared in a similar way[28] from the respective orthoester **7**[29] or **8**[30] and naphthyl-2-thiol. The acetate in position 2 was replaced by a benzoate in two steps to give **5** and **6**. Compound **14** was obtained in two steps from **5** and ethyl 6-hydroxy hexanoate. The naphthalene-2-thiol is a commercially available aromatic thiol, it is crystalline, almost odorless and less toxic than thiophenol, previously used for mannoside preparation. Furthermore, we recently proposed 2-methyl-5-*tert*-butylthiophenol as an improved thiophenol surrogate.[31]

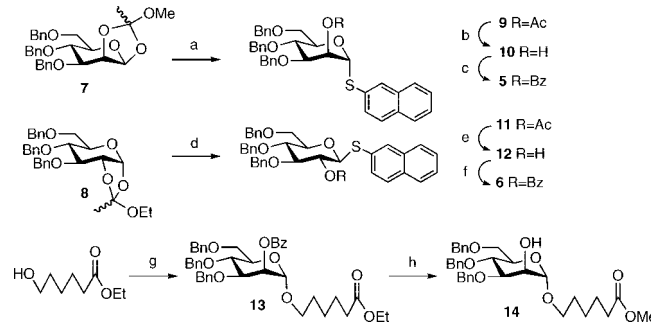
The iterative strategy for the α mannoside is depicted in Scheme 4. The synthesis was uneventful and the overall yield for the introduction of an α manno unit was good (60−78%).

Scheme 2. Iterative Synthesis of β (1→2) Oligomannosides Repeated until the Tetrasaccharide **29** is Obtained[a]



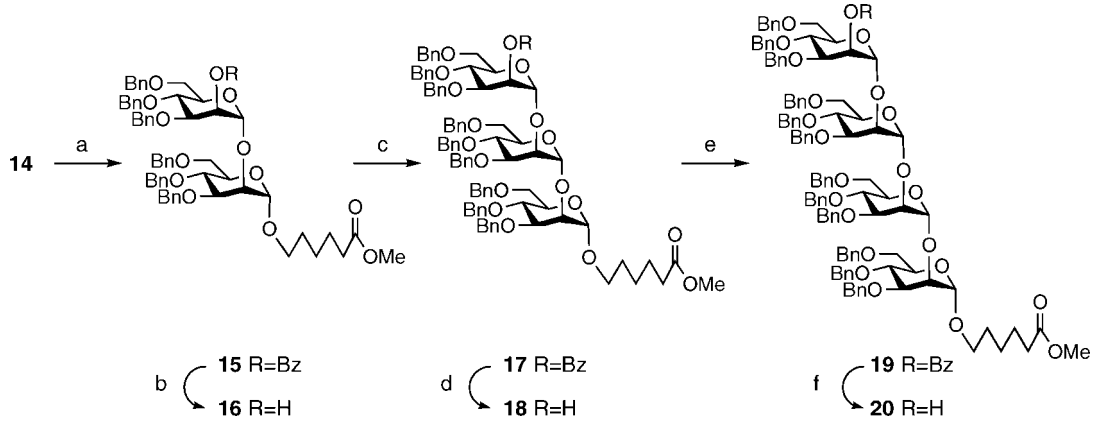
[a] Reagents and conditions: (a) **6**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, rt; (b) MeONa, MeOH; (c) Swern oxidation; (d) $NaBH_4$, MeOH.

Scheme 3[a]



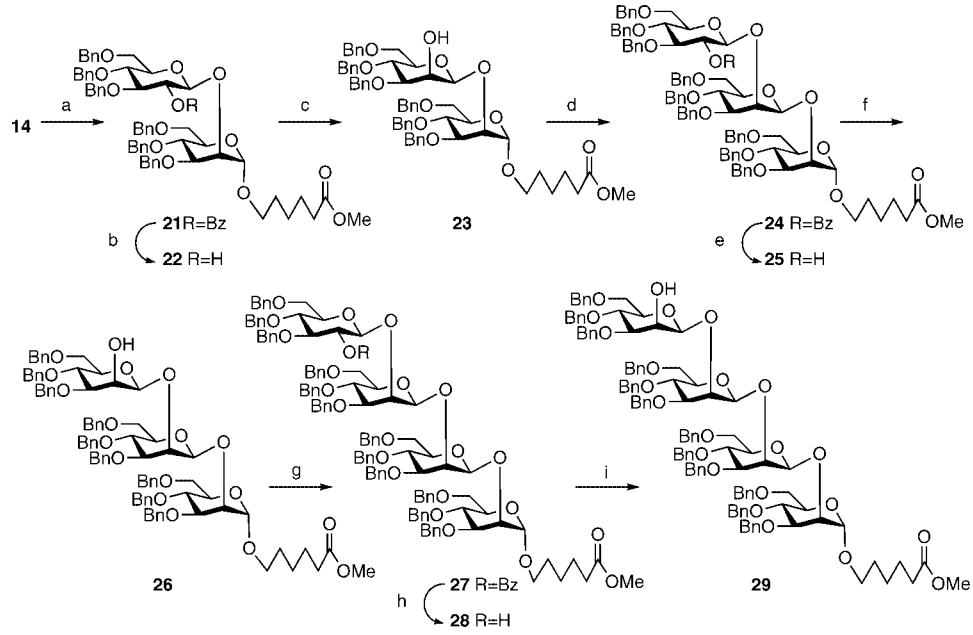
[a] Reagents and conditions: (a) naphthalene-2-thiol, $HgBr_2$, (0.1 equiv), CH_3CN, 60 °C, 24 h, 69%; (b) MeONa, MeOH, rt, 24 h, 80%; (c) BzCl, NEt_3, DMAP, CH_2Cl_2, rt, 17 h, 90%; (d) naphthalene-2-thiol, $HgBr_2$, (0.1 equiv), CH_3CN, 60 °C, 24 h, 63%; (e) MeONa, MeOH, rt, 18 h, 71%; (f) BzCl, NEt_3, DMAP, CH_2Cl_2, rt, 36 h, 91%; (g) **5**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, rt, 0.5 h, 64%; (h) MeONa, MeOH, rt, 3 h, 91%.

Scheme 4[a]



[a] Reagents and conditions: (a) **5**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, rt, 79%; (b) MeONa, MeOH, 77%; (c) **5**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 77%; (d) MeONa, MeOH, 87%; (e) **5**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 95%; (f) MeONa, MeOH, 82%.

Scheme 5[a]



[a] Reagents and conditions: (a) **6**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 87%; (b) MeONa, MeOH, 90%; (c) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, then MeOH, $NaBH_4$, 79%; (d) **6**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 78%; (e) MeONa, MeOH, 91%; (f) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, then $NaBH_4$, MeOH (15% Manno + 70% Gluco **25**); (g) **6**, NIS, TfOH, 4 Å molecular sieves, CH_2Cl_2, 87%; (h) MeONa, MeOH, 86%; (i) oxalyl chloride, DMSO, NEt_3, CH_2Cl_2, then $NaBH_4$, MeOH (15% Manno + 63% Gluco **28**).

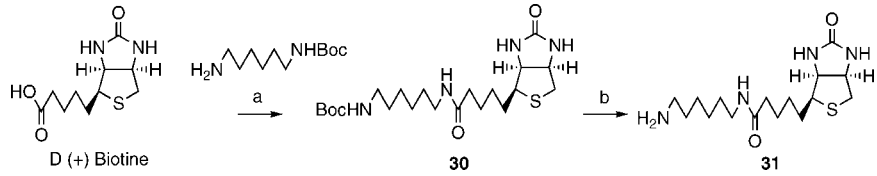
The iterative strategy for the β-mannoside is depicted in Scheme 5. The selectivity of the reduction step is rather surprising, although the oxidation−reduction steps gave the dimannoside with a total *manno* selectivity, this sequence led predominantly the *gluco* isomer on the tri and tetrasaccharide level. This is in contrast with our previous work[26a,b] but using a different reducing agent, superhydride, different temperature, and aglycon. This length-dependent behavior is probably due to the helical structure of the β (1→2) mannoside (the step is ca. four mannose units[24c]) as the reduction of the disaccharide is completely selective. At this stage, recycling the gluco compounds allowed enough compound to be available for the subsequent biotinylation step. The use of an alternative reducing agent for this transformation is underway.

Biotinylation. The protected oligomannosides **16**, **20**, **23**, and **29** were then coupled to the amine **31**. The amine **31**[32] was first prepared in two steps from biotin (Scheme 6). The free base of **31** was obtained from the trifluoroacetate salt upon treatment with an ion-exchange resin.

The methyl esters **16**, **20**, **23**, and **29** were saponified with sodium hydroxide in aqueous THF (Scheme 7). The acids **32**−**35** were then condensed with the amine **31** in DMF in the presence of EDC (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride) to give the protected biotinylated compounds (**36**−**39**). Oxidation (*m*-chloroperbenzoic acid) of these biotin conjugates followed by hydrogenolysis of the benzyl group (H_2, 1.3 atm) allowed access to the final products (**1**−**4**) without any difficulties owing to the inertness of the biotin sulfone group. The yields are summarized in Table 1.

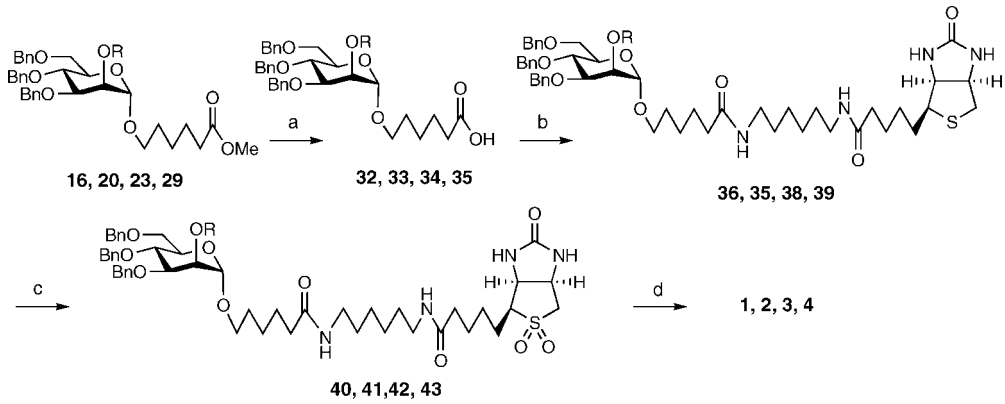
Alternatively, in a more direct strategy, we prepared and tried to use compound **45**, the sulfone form of **31** (Scheme 8). However, this compound was insoluble in most of the solvents and the coupling reaction failed. It was, therefore, more

Scheme 6[a]



[a] Reagents and conditions: (a) EDC, pyridine, 60 °C, 91%; (b) CF_3COOH, ultrasonic irradiation, then Amberlite IRA-67, 100%.

Scheme 7[a]



[a] Reagents and conditions: (a) NaOH, THF, H_2O; (b) **31**, EDC, DMAP, DMF; (c) mCPBA, CH_2Cl_2; (d) H_2, Pd/C, MeOH/H_2O.

Table 1. Yields of Final Steps

	(a) saponification	(b) biotinylation	(c) sulfur oxidation	(d) benzyl hydrogenolyse
1 Di α	**16** → **32** (96%)	→ **36** (78%)	→ **40** (82%)	→ **1** (89%)
2 Tetra α	**20** → **33** (82%)	→ **37** (68%)	→ **41** (84%)	→ **2** (90%)
3 Di β	**23** → **34** (98%)	→ **38** (79%)	→ **42** (81%)	→ **3** (90%)
4 Tetra β	**29** → **35** (95%)	→ **39** (71%)	→ **43** (94%)	→ **4** (90%)

preferable to perform the coupling reaction using the more lipophilic sulfide form.

3. Immunological Evaluation of the Conjugates

Monoclonal Antibodies (Mabs). Synthetic oligomannosides were tested with a panel of anti-*C. albicans* glycan Mabs, whose specificity was established previously. Mab anti-β (1→3) glucan was used as a control. Mabs EB CA1, a rat IgM, was described as reacting with an α (1→2) mannopentaose as a minimal epitope.[33] Mab 5B2, a rat−mouse hybrid IgM, was described as reacting with β (1→2) mannosides with a mannobiose as a minimal epitope.[34,35] Mab B6.1, a mouse IgM, has been described as specific for a β (1→2) mannotriose.[22] Mab EBA2, reacting with a galactofuranose epitope, was used as a control.

Multiplex Quantification of Biotin Sulfone Tagged Oligomannoside (BSTO) Reactivity with Anticarbohydrate Monoclonal Antibodies. A total of four BSTO epitopes were individually coupled to different streptavidin coated microsphere beads and tested with a panel of anti-*C. albicans* Mabs directed against mannoglycoconjugates (EB CA1, 5B2, and B6.1) or β (1→3) glucans (Biosupplies). Figure 2 represents the reactivity of individual Mabs with the panel of BSTO in multiplex assay. The antigalactofuran Mab did not generate any signal with any of the mannose residues presented as BSTOs. As expected, Mab 5B2 bound to the members of the β mannose family but did not bind to any α mannose. As also expected, its reactivity with this family started with the mannobiose. As also expected, Mab B6.1 did not react with β-mannobiose **3** although it did react with β-mannotetraose **4**. As for 5B2, no reactivity was observed against α-mannosides **1** or **2**, confirming the strong specificity conferred by the anomeric configuration. In the vein, Mab EBCA1, described as reacting with an α-mannopentaoside, bound to some extent to the α-mannotetraoside, which was in our series of BSTOs the closest epitope. Importantly, no reactivity with members of the β family was observed.

Surface Plasmon Resonance Analysis of Biotin Sulfone Tagged Oligomannoside (BSTO) Reactivity with Anticarbohydrate Monoclonal Antibodies. The typical results for the interaction between synthetic oligomannosides and antibodies are shown in Figure 3. No binding was observed for di α mannoside **1**. The variations of about 5 RU for each Mab are negligible, and modifications of sensorgramm profiles can be attributed to composition buffer variations. The presence of ligand (i.e., **1**) was confirmed by the binding of Concanavalin A (250 nM) on this support (data not shown). The EB CA1 Mab weakly interacted with tetra α mannoside **2** (about 60 RU) and presented a typical profile of a sensorgramm with association and dissociation curves (beginning respectively after 60 and 180 s). EB A2, B6.1, and 5B2 antibodies did not show any interactions with **2**, which is not surprising considering the specificity of each antibody.

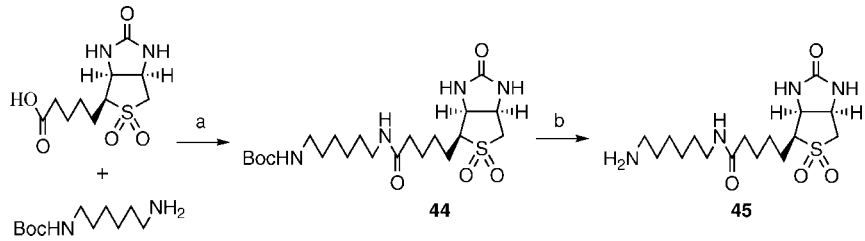
Interactions of antibodies with β-oligomannosides **3** and **4** were more informative. 5B2 interacted with di-β mannoside **3** (about 600 RU) and the tetra-β mannoside **4**. B6.1 showed strong binding only to the tetra-β mannoside **4**. The absence of a response for EB A2 injections shows the specificity of the binding.

These experiments are in complete agreement with the known specificity of the anti-*C. albicans* Mabs and are consistent with the microsphere measurements obtained by multiple-analyte profiling technology (Luminex).

4. Conclusions

Extensive studies on the interaction between *C. albicans* and its hosts have suggested that the spacial conformation of β-mannoses versus α-mannoses represents the support for differential recognition of *C. albicans* mannoglycoconjugates

Scheme 8[a]



[a] Reagents and conditions: (a) EDC, pyridine, 60 °C, 15 h, 91%; (b) CF_3COOH, 5 min, rt, 100%.

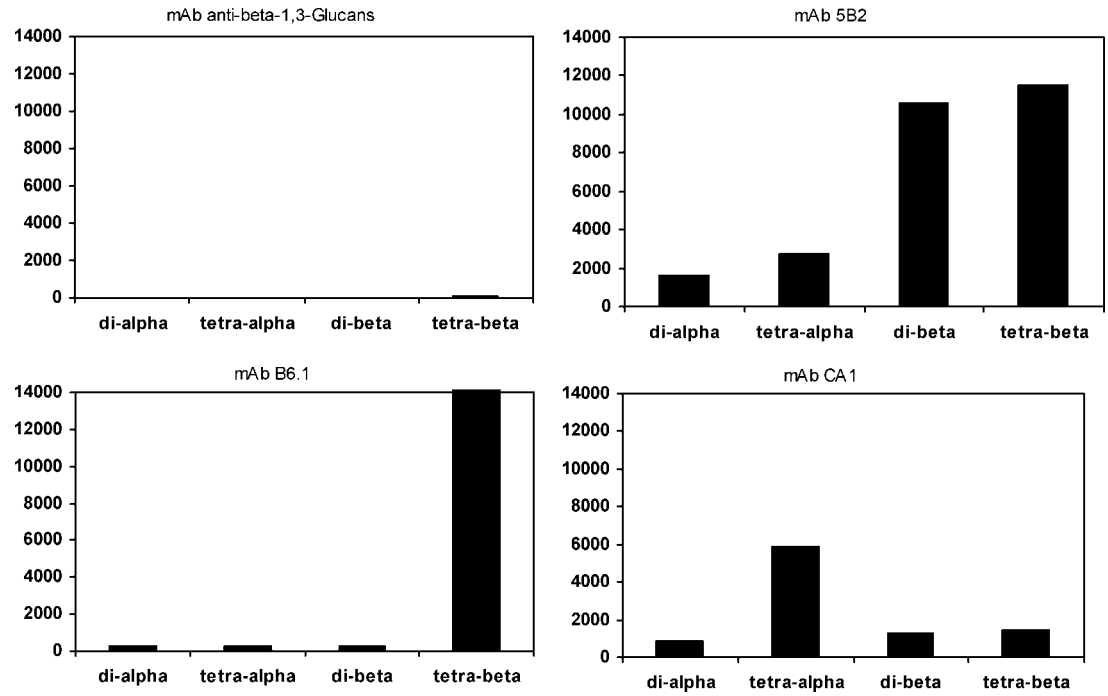
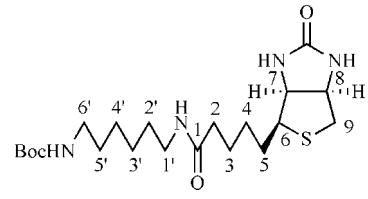


Figure 2. Multiplex quantification of biotin sulfone tagged oligomannoside (BSTO) reactivity with anticarbohydrate monoclonal antibodies.

by the mammalian immune system. This has been observed for lectins of innate immunity where β-mannosides have been shown to react with galectin-3, whereas α-mannosides react with a large number of C-lectins.[15,23−25] Interaction with these receptors directs the balance between pro- and anti-inflammatory responses, conditioning the damage to the host.[15] This strong duality in reactivity is also found for adaptative immunity because the so-called antimannan antibodies generated after *C. albicans* infection or immunization distribute between anti-β-mannosides, which are "protective" for the host, and anti-α-mannosides, which are not.[17] To make progress in the understanding of this complex interplay, it is essential to dispose of reliable and easy tools for large scale dissection of the antibody response. In the present study, we used representative members of anti-α man and anti-β man antibodies to validate the new technology we have developed. This development was intended to present molecules to any kind of support that could be sensitized with avidin. Using versatile multiplex technology, as well as the more analytical BIAcore technology, we found that the BTSO reproduced and refined in simple runs data hardly accumulated in the literature by EIA and Western blot testing of yeasts derived oligomannosides preparations.

Experimental Section

General Procedures. All compounds were homogeneous by TLC analysis and had spectral properties consistent with their assigned structures. Melting points were determined in capillary tubes in a Büchi 510 apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer model 241 digital polarimeter at 22 ± 3 °C. Compound purity was checked by TLC on Silica gel 60 F_{254} (E. Merck) with detection by charring with sulfuric acid. Column chromatography was performed on Silica gel 60 (E. Merck). ^{1}H NMR spectra were recorded with Brüker AM 250, AM 400 instruments. Chemical ionization and FAB mass spectrometry were recorded with Jeol MS700: CI (gas: ammonia); FAB (matrix: NBA, NaI).



Typical Hydrogenolysis Procedure. Conjugate 1. To a solution of 40 (275 mg, 0.201 mmol) in anhydrous methanol (15 mL) was added Pd/C (10%) (137 mg, 0.5 g/g of substrate). Vacuum and H_2 were alternated, and then the mixture was stirred at room temperature under H_2 overnight. The mixture was filtered through celite and concentrated. The residue was dissolved in water (HPLC grade) and washed with CH_2Cl_2 (three times). The aqueous layer was filtered over PTFE 0.45 μm filter and lyophilized to give 1 (56 mg, 82%) as a white solid. $[\alpha]^{25}_D$ +48 (c 1.0, MeOH/H_2O (1/1)). Anal. ($C_{34}H_{60}O_{16}N_4S$) C, H.

Conjugate 2. White solid. Yield: 90%. $[\alpha]^{25}_D$ +44 (c 0.4, MeOH/H_2O (1/1)). Anal. ($C_{46}H_{80}N_4O_{26}S$) C, H.

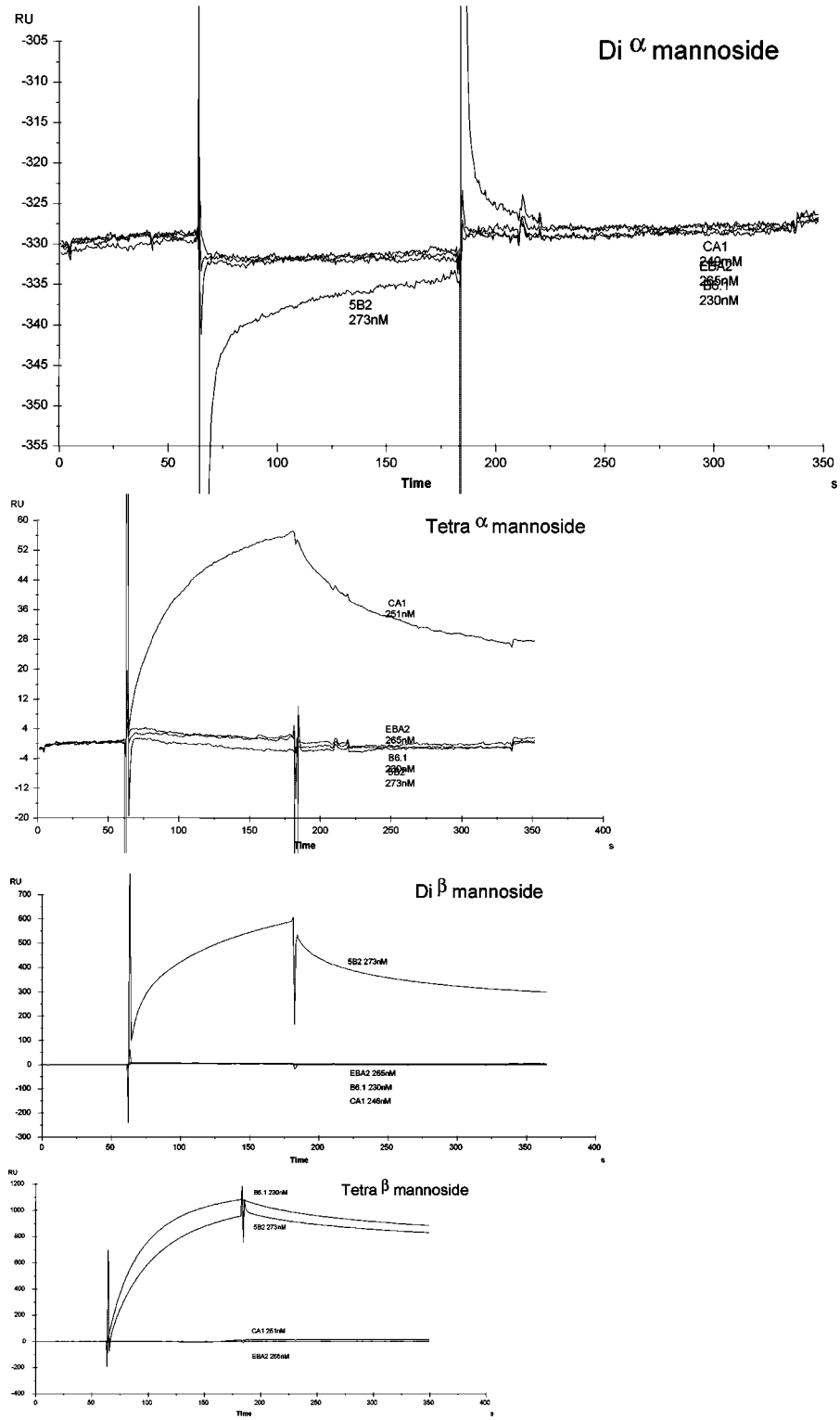


Figure 3. Surface plasmon resonance analysis.

Conjugate 3. Debenzylation of **42**. White solid. Yield: 90%. $[\alpha]^{25}_D$ +10 (c 0.5, MeOH/H_2O (1/1)). Anal. ($C_{34}H_{60}O_{16}N_4S$) C, H.

Conjugate 4. Debenzylation of **43**. White solid. Yield: 90%. $[\alpha]^{25}_D$ −4.8 (c 1.2, MeOH/H_2O (1/1)). Anal. ($C_{46}H_{80}N_4O_{26}S$) C, H.

2-Naphthyl 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (5). To a mixture of **10** (0.356 g, 0.600 mmol) and DMAP (16.2 mg, 0.133 mmol, 0.2 equiv) in anhydrous CH_2Cl_2 (5 mL) were added at 0 °C, under argon: triethylamine (0.59 mL, 4.20 mmol, 7.0 equiv) and benzoyl chloride (90.5 μL, 0.780 mmol, 1.3 equiv). The mixture was stirred under argon for 17 h and then washed with HCl (1 M). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 9/1) to give **5** (0.376 g, 90%) as a syrup. R_f: 0.70 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +10 (c 1.0, $CHCl_3$).

2-Naphthyl 2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-1-thio-*β*-D-glucopyranoside (6). To a mixture of **12** (6.26 g, 10.57 mmol) and DMAP (0.64 g, 5.28 mmol, 0.5 equiv) in anhydrous CH_2Cl_2 (80 mL) were added at 0 °C under argon: triethylamine (7.35 mL, 26.43 mmol, 5.0 equiv) and benzoyl chloride (6.13 mL, 26.43 mmol, 5.0

equiv). The mixture was stirred under argon for 36 h and then washed with HCl (1 M). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in CH_2Cl_2, and the product was precipitated by addition of cyclohexane to give **6** (6.380 g, 91%) as a white solid. R_f: 0.54 (cyclohexane/EtOAc: 2/1); mp: 112 °C. $[\alpha]^{25}_D$ +29 (c 1.0, $CHCl_3$).

2-Naphthyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (9). To a mixture of orthoester **7** (3.32 g, 6.56 mmol) and naphthalene-2-thiol (3.48 g, 21.7 mmol, 3.3 equiv) in anhydrous CH_3CN (10 mL) was added mercury bromide (0.296 g, 0.82 mmol, 0.1 equiv) under argon. The mixture was stirred at 60 °C for 27 h. The mixture was allowed to cool to room temperature, diluted with EtOAc, and washed with NaOH (5%) and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 9/1) to give **9** (2.85 g, 69%) as a white solid. R_f: 0.50 (cyclohexane/EtOAc: 8/2); mp: 82 °C. $[\alpha]^{25}_D$ +14 (c 1.0, $CHCl_3$).

2-Naphthyl 3,4,6-Tri-*O*-benzyl-1-thio-α-D-mannopyranoside (10). To a solution of **9** (2.30 g, 3.63 mmol) in methanol (50 mL), was added sodium (1.6 g, 72.5 mmol, 20.0 equiv). The mixture was stirred for 22 h and then neutralized (IR-120 H^+ resin), filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8/2) to give **10** (1.070 g, 50%) as a syrup. R_f: 0.20 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +20 (c 1.0, $CHCl_3$).

2-Naphthyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (11). To a mixture of orthoester **8** (10.1 g, 12.6 mmol) and naphthalene-2-thiol (6.68 g, 41.7 mmol, 3.3 equiv) in anhydrous CH_3CN (32 mL), was added mercury bromide (0.227 g, 0.630 mmol, 0.05 equiv) under argon. The mixture was stirred at 60 °C for 23 h. After the mixture was cooled to room temperature, it was diluted with EtOAc and washed with NaOH (5%) and water. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 9/1) to give **11** (5.06 g, 63%) as a white solid. R_f: 0.5 (cyclohexane/EtOAc: 3/1); mp: 125 °C. $[\alpha]^{25}_D$ +10 (c 1.0, $CHCl_3$).

2-Naphthyl 3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (12). To a solution of **11** (0.185 g, 0.291 mmol) in methanol (15 mL) was added sodium (0.324 g, 14.1 mmol, 48 equiv). The mixture was stirred for 18 h and then neutralized (IR-120 H^+ resin), filtered and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8/2) to give **12** (0.122 g, 71%) as a white solid. R_f: 0.41 (cyclohexane/EtOAc: 3/1); mp: 94 °C. $[\alpha]^{25}_D$ −6 (c 1.0, $CHCl_3$).

5-Ethoxycarboxypentyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (13). To a mixture of **4** (3.000 g, 4.31 mmol), molecular sieves 4Å (3 g) in anhydrous CH_2Cl_2 were added successively under argon: ethyl 6-hydroxy-hexanoate (1.050 mL, 6.460 mmol, 1.5 equiv), NIS (2.420 g, 10.770 mmol, 2.5 equiv), and TfOH (0.191 mL, 2.150 mmol, 0.5 equiv). After 30 min, the precipitate was filtered off, and the filtrate was successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 9/1 to 8/2) to give **13** (1.910 g, 64%) as a syrup. R_f: 0.45 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ −7 (c 0.7, $CHCl_3$).

Typical Debenzoylation Procedure. 5-Methoxycarbonylpentyl 3,4,6-Tri-*O*-benzyl-α-D-mannopyranoside (14). To a solution of **13** (1.910 g, 2.744 mmol) in methanol (30 mL) was added sodium (0.012 g, 0.548 mmol, 0.2 equiv). The mixture was stirred for 3 h and then neutralized (IR-120 H^+ resin), filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 7/3) to give **14** (1.480 g, 91%) as a syrup. R_f: 0.60 (cyclohexane/EtOAc: 1/1). $[\alpha]^{25}_D$ +36 (c 0.8, $CHCl_3$).

Typical Glycosylation Procedure. 5-Methoxycarbonylpentyl 2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (15). To a mixture of **14** (0.102 g, 0.176 mmol) and **5** (0.184 g, 0.264 mmol, 1.5 equiv) and 4Å molecular sieves (0.100 g) in anhydrous CH_2Cl_2 (5 mL), were added under argon NIS (0.099 g, 0.440 mmol, 2.5 equiv) and TfOH (0.008 mL, 0.088 mmol, 0.5 equiv). After 30 min, the precipitate was filtered off, and the filtrate was successively washed with saturated aqueous $Na_2S_2O_3$, $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 9/1 to 8/2) to give **15** (0.156 g; 79%) as a syrup. R_f: 0.35 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +1 (c 1.0, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (16). Debenzoylation of **15**. Column chromatography cyclohexane/EtOAc: 8/2 to 7/3. Syrup. Yield: 97%. R_f: 0.65 (cyclohexane/EtOAc: 1/1). $[\alpha]^{25}_D$ +34 (c 0.5, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (17). Glycosylation of **16** with **5**. Column chromatography (cyclohexane/EtOAc: 9/1 to 8/2). Syrup. Yield: 77%. R_f: 0.35 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +12 (c 1.0, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (18). Debenzoylation of **17**. Column chromatography (cyclohexane/EtOAc: 8/2). Syrup. Yield 77%. R_f: 0.15 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +32 (c 0.6, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (19). Glycosylation of **18** with **5**. Column chromatography (cyclohexane/EtOAc: 8/2). Syrup. Yield: 95%. R_f: 0.30 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ +20 (c 0.4, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (20). Debenzoylation of 19. Column chromatography (cyclohexane/EtOAc: 7/3). Syrup. Yield: 87%. R_f: 0.20 (cyclohexane/EtOAc: 9/1). $[\alpha]^{25}_D$ +38 (c 0.4, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-1-*O*-α-D-mannopyranoside (21). Glycosylation of **14** and **6**. Column chromatography (cyclohexane/EtOAc: 8/2). Syrup. Yield: 87%. R_f: 0.44 (cyclohexane/EtOAc: 2/1). $[\alpha]^{25}_D$ +5 (c 0.5, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*d*-mannopyranoside (22). Debenzoylation of **21**. Column chromatography (cyclohexane/EtOAc: 8/2. Syrup. Yield: 90%). R_f: 0.35 (cyclohexane/EtOAc: 2/1). $[\alpha]^{25}_D$ +9 (c 1.0, $CHCl_3$).

Typical Swern Oxidation/Reduction Procedure. 5-Methoxycarbonylpentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (23). To a solution of oxalyl chloride (1.497 mL, 17.424 mmol, 3 equiv) in anhydrous CH_2Cl_2 (10 mL) cooled at −78 °C was added dropwise DMSO (2.474 mL, 34.848 mmol, 6 equiv). After 15 min, a solution of **22** (5.972 g, 5.808 mmol, 1 equiv) in CH_2Cl_2 (20 mL) was added dropwise and the mixture was stirred for a further 45 min at −78 °C. Triethylamine (4.888 mL, 34.848 mmol, 6 equiv) was then added dropwise, and the mixture was allowed to warm up to room temperature. After 30 min, the mixture was extracted with CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$, filtered, and diluted with an equivalent volume of methanol. $NaBH_4$ (0.658 g, 17.424 mmol, 3 equiv) was then added, and the solution was degassed. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 8/2 to 7/3) to give **23** (4.681 g, 79%) as a syrup. Chromatography (cyclohexane/EtOAc: 7/3). Syrup. Yield: 79%. R_f: 0.32 (cyclohexane/EtOAc: 2/1) $[\alpha]^{25}_D$ +0.6 (c 5.0, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (24). Glycosylation of **23** with **6**. Column chromatography (cyclohexane/

EtOAc: 8/2). Syrup. Yield: 78%. R_f: 0.47 (cyclohexane/EtOAc: 8/2). $[\alpha]^{25}_D$ −15 (c 1.7, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (25). Debenzoylation of **24**. Column chromatography (cyclohexane/EtOAc: 8/2 to 7/3). Syrup. Yield: 91%. R_f: 0.41 (cyclohexane/EtOAc: 7/3). $[\alpha]^{25}_D$ −34 (c 3.6, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (26). Oxidation/reduction of **25**. Column chromatography (cyclohexane/EtOAc: 8/2 to 7/3). Syrup. Yield: 15%; 70% starting material **25** recovered. R_f: 0.32 (cyclohexane/EtOAc: 7/3). $[\alpha]^{25}_D$ −34 (c 1.0, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (27). Glycosylation of **26** with **6**. Column chromatography (cyclohexane/EtOAc: 8/2). Syrup. Yield: 87%. R_f: 0.47 (cyclohexane/EtOAc: 7/3). $[\alpha]^{25}_D$ −46 (c 1.4, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (28). Debenzoylation of **27**. The mixture was refluxed for 2 h. Column chromatography (cyclohexane/EtOAc: 7/3). Syrup. Yield: 86%. R_f: 0.41 (cyclohexane/EtOAc: 7/3). $[\alpha]^{25}_D$ −42.5 (c 1.2, $CHCl_3$).

5-Methoxycarbonylpentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (29). Oxidation/reduction of **28**. Column chromatography (cyclohexane/EtOAc: 8/2 to 7/3). Syrup. Yield: 15%; 63% starting material recovered. R_f: 0.37 (cyclohexane/EtOAc: 7/3). $[\alpha]^{25}_D$ −43.2 (c 1.3, $CHCl_3$).

(3a*S*,4*S*,6a*R*)-*N*-[6-[(1,1-Dimethylethoxy)carbonyl]aminohexyl]hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanamide (30). To a mixture of D-(+) biotin (0.900 g, 3.68 mmol) and *N*-Boc-1,6-diaminohexane hydrochloride (0.929 g, 3.68 mmol, 1 equiv) in anhydrous pyridine (30 mL) was added, under argon, EDC (0.843 g, 4.41 mmol, 1.2 equiv). The mixture was stirred overnight and concentrated, the crude product was filtered and washed with NaOH (1 M) and ether. The product was dried under vacuum to give **30** (1.448 g, 91%) as a white powder. R_f: 0.44 (CH_2Cl_2/MeOH: 9/1); mp: 173.7 °C. $[\alpha]^{25}_D$ +38 (c 1.0, MeOH).

(3a*S*,4*S*,6a*R*)-*N*-(6-Aminohexyl)hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanamide (31). 30 (0.614 g, 1.390 mmol) was dissolved in trifluoroacetic acid (5 mL) and sonicated until total dissolution. After 5 min, the TFAH was evaporated. The residue was dissolved in methanol, neutralized (Amberlite IRA-67), and filtered. The methanol was evaporated to give **31** (0.476 g, 100%) as a syrup.

Typical Saponification Procedure. 5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (32). To a solution of **16** (551 mg, 0.544 mmol) in THF (10 mL) was added NaOH (1 M, 3.26 mL). The mixture was stirred at 60 °C for 24 h, neutralized (IR-120 H^+ resin), filtered, and concentrated. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc: 5/5, →acetone) to give **32** (520 mg, 96%) as a syrup. R_f: 0.38 (cyclohexane/EtOAc: 5/5). $[\alpha]^{25}_D$ +36 (c 0.5, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (33). Column chromatography (cyclohexane/EtOAc: 5/5 → 100% EtOAc). Syrup; yield: 82%. R_f: 0.58 (cyclohexane/EtOAc: 6/4).

5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (34). Saponification of **23**. Column chromatography (cyclohexane/EtOAc: 5/5 to 100% EtOAc). Syrup; yield: 98%. R_f: 0.38 (cyclohexane/EtOAc: 1/1). $[\alpha]^{25}_D$ −3 (c 0.9, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (35). Saponification of **29**. Column chromatography (cyclohexane/EtOAc: 5/5, 100% acetone). Syrup; yield: 95%. R_f: 0.55 (cyclohexane/EtOAc: 5/5). $[\alpha]^{25}_D$ −43.4 (c 1.7, $CHCl_3$).

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Typical Biotin Coupling Procedure. 5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Biotin Conjugate (36). To a mixture of **32** (500 mg, 0.501 mmol) and **31** (1.5 equiv) in anhydrous DMF (10 mL), were added DMAP (73 mg, 0.601 mmol, 1.2 equiv) and EDC (143 mg, 0.752 mmol, 1.5 equiv). The mixture was stirred at 60 °C overnight. EDC (95 mg, 1 equiv) was then added to complete the reaction. The solution was concentrated, and the residue was extracted with CH_2Cl_2, washed with water, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: 9/1) to give **36** (521 mg, 78%) as a syrup. R_f: 0.5 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +32 (c 1.6, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Biotin Conjugate (37). Column chromatography (CH_2Cl_2/MeOH: 10/1 → 9/1). Syrup; yield: 68%. R_f: 0.53 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +25 (c 1.7, $CHCl_3$).

5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Biotin Conjugate (38). Coupling of **34** with **31**. Column chromatography (CH_2Cl_2/MeOH: 9/1). Syrup; yield: 79%. R_f: 0.51 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +6 (c 2.8, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Biotin Conjugate (39). Coupling of 35 with 31. Column chromatography (EtOAc/acetone/MeOH: 6/3/1). Syrup; yield: 71%. R_f: 0.47 (EtOAc/Acetone/MeOH: 6/3/1). $[\alpha]^{25}_D$ −30.5 (c 1.1, $CHCl_3$).

Typical Biotin Oxidation Procedure. 5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Oxidized Biotin Conjugate (40). To a solution of **36** (455 mg, 0.344 mmol) in anhydrous CH_2Cl_2 (15 mL) was added *m*-chloroperbenzoic acid (178 mg, 1.034 mmol, 3 equiv) under argon. The mixture was stirred at room temperature overnight, washed with a saturated aq $NaHCO_3$, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH: 9/1) to give **40** (384 mg, 82%) as a syrup. R_f: 0.60 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +24 (c 1.2, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Oxidized Biotin Conjugate (41). Column chromatography (CH_2Cl_2/MeOH: 9/1). Syrup; yield: 84%. R_f: 0.44 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +24 (c 1.2, $CHCl_3$).

5-Carboxypentyl 2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Oxidized Biotin Conjugate (42). Oxidation of **38**. Column chromatography CH_2Cl_2/MeOH: 9/1. Syrup; yield: 81%. R_f: 0.42 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ +5 (c 1.7, $CHCl_3$).

5-Carboxypentyl 2-*O*-(2-*O*-(2-*O*-(3,4,6-Tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-β-D-mannopyranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside Oxidized Biotin Conjugate (43). Oxidation of **39**. Column chromatography (CH_2Cl_2/MeOH: 9/1). Syrup; yield: 94%. R_f: 0.39 (CH_2Cl_2/MeOH: 9/1). $[\alpha]^{25}_D$ −23.7 (c 1.3, $CHCl_3$).

Compound (44). To a mixture of biotin sulfone (0.478 g, 1.731 mmol) and *N*-Boc-1,6-diaminohexane hydrochloride (0.436 g, 1.731 mmol, 1 equiv) in anhydrous pyridine (18 mL) was added, under

argon, EDC (0.792 g, 4.154 mmol, 2.4 equiv). The mixture was allowed to stir overnight at 60 °C. The solvent was evaporated, and the crude product was filtered and washed with CH_2Cl_2, HCl (1 M), and acetone. The product was dried under vacuum to give 0.748 g (91%) as a white powder. R_f: 0.55 ($CHCl_3$/MeOH: 5/5); mp: 237 °C.

Compound (45). 44 (0.100 g, 0.211 mmol) was dissolved in trifluoroacetic acid (2 mL) and sonicated until total dissolution. After 5 min, the TFA was evaporated. The residue was dissolved in methanol, neutralized (Amberlite IRA-67), and filtered. The methanol was evaporated to give 0.080 g (100%) as a syrup.

Monoclonal Antibodies (Mabs). Synthetic oligomannosides were tested with a panel of anti-*C. albicans* glycan Mabs, whose specificity was established previously. Mab anti-β (1→3) glucan (Biosupplies, Australia) was used as a control. Mabs EB CA1, a rat IgM (Bio-Rad Laboratories, France), was described as reacting with an α (1→2) mannopentaose as a minimal epitope.[33] Mab 5B2, a rat−mouse hybrid IgM, was described as reacting with β (1→2-mannosides with a mannobiose as a minimal epitope.[34,35] Mab B6.1, a mouse IgM, has been described as specific for a β (1→2) mannotriose.[22] Mab EBA2, reacting with a galactofuranose epitope, was used as a control (Bio-Rad).

Coupling of Biotin Sulfone Tagged Oligomannosides to Fluorescent Magnetic Beads. Polystyrene fluoromagnetic beads were prepared via a four-step procedure. (i) Carboxyl groups on the surface of the beads were activated in the presence of *N*-hydroxysuccinimide and EDC (Pierce Chemicals Co. Rockford, Ill, USA) to form activated esters. (ii) After three washes in 50 mM phosphate buffered saline (PBS), pH 7.4, these esters were reacted for 3 h at room temperature with avidin (Sigma, Saint Quentin Fallavier, France). (iii) The beads were blocked for 30 min with 50 mM PBS containing 250 mM NH_2OH. (iv) After three washes in PBS, the beads were coupled with biotin sulfone tagged oligomannosides for 1 h at room temperature and blocked overnight with 50 mM PBS containing 10% bovine serum albumin. After washing, the beads were stored at 4 °C.

Multiplex Quantification of Biotin Sulfone Tagged Oligomannoside (BSTO) Reactivity with Anticarbohydrate Monoclonal Antibodies. Mixed suspensions of microspheres were allowed to react with each Mab diluted 1/200 for 30 min at 37 °C in PBST under agitation. After three washes in PBST, the microspheres were incubated with appropriate anti-immunoglobulins coupled to phycoerythrin (Southern Biotech, USA). After three washes in PBST, the microspheres were resuspended in PBST in a test tube and the reaction was monitored on a Luminex Laboratory MAP system 100 (Luminex USA) at 532 nm. The results are expressed as median fluorescence intensity determined for 100 microspheres of each BSTO identified by its microsphere number.

Surface Plasmon Resonance Analysis of Biotin Sulfone Tagged Oligomannoside (BSTO) Reactivity with Anticarbohydrate Monoclonal Antibodies. BIAcore 3000 instrument, BIAevaluation software 3.0, and sensor chip SA (streptavidin) were obtained from BIAcore (GE Healthcare). BSTOs were fixed on the flow cells at a 5 nM concentration in HBS buffer according to the manufacturer's recommendations. The level of bound ligands was approximately 25−30 response units (RU). The five Mabs were injected at a 250 nM concentration after HBS dilution to 30 μL/min over a 2 min period. The regeneration step was performed with a 250 mM NaCl, 10 mM NaOH buffer. A reference flow cell (i.e., flow cell without ligand) was used for each ligand. Quantification of specific binding was obtained from the difference between the ligand and reference response.

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Supporting Information Available: NMR and mass spectroscopy characterization data for the synthesis of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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