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Endolysins of *Bacillus anthracis* Bacteriophages Recognize Unique Carbohydrate Epitopes of Vegetative Cell Wall Polysaccharides with High Affinity and Selectivity

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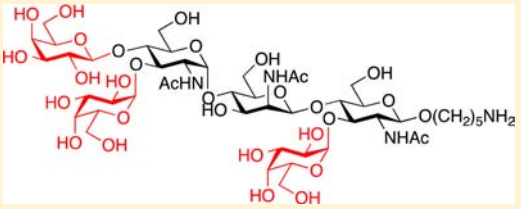
Supporting Information

ABSTRACT: Bacteriophages express endolysins which are the enzymes that hydrolyze peptidoglycan resulting in cell lysis and release of bacteriophages. Endolysins have acquired stringent substrate specificities, which have been attributed to cell wall binding domains (CBD). Although it has been realized that CBDs of bacteriophages that infect Gram-positive bacteria target cell wall carbohydrate structures, molecular mechanisms that confer selectivity are not understood. A range of oligosaccharides, derived from the secondary cell wall polysaccharides of *Bacillus anthracis*, has been chemically synthesized. The compounds contain an α-D-GlcNAc-(1→4)-β-D-ManNAc-(1→4)-β-D-GlcNAc backbone that is modified by various patterns of α-D-Gal and β-D-Gal branching points. The library of compounds could readily be prepared by employing a core trisaccharide modified by the orthogonal protecting groups N^{α}-9-fluorenylmethyloxycarbonate (Fmoc), 2-methylnaphthyl ether (Nap), levulinoyl ester (Lev) and dimethylthexylsilyl ether (TDS) at key branching points. Dissociation constants for the binding the cell wall binding domains of the endolysins PlyL and PlyG were determined by surface plasmon resonance (SPR). It was found that the pattern of galactosylation greatly influenced binding affinities, and in particular a compound having a galactosyl moiety at C-4 of the nonreducing GlcNAc moiety bound in the low micromolar range. It is known that secondary cell wall polysaccharides of various bacilli may have both common and variable structural features and in particular differences in the pattern of galactosylation have been noted. Therefore, it is proposed that specificity of endolysins for specific bacilli is achieved by selective binding to a uniquely galactosylated core structure.



INTRODUCTION

Bacillus anthracis is a Gram-positive, spore-forming bacterium that causes anthrax in humans and other mammals.[1] The relative ease by which *B. anthracis* can be weaponized and difficulties associated with the early recognition of inhalation of anthrax due to the nonspecific nature of its symptoms were underscored by the deaths of five people who inhaled spores from contaminated mail.[2] As a result, there is a renewed interest in anthrax vaccines and early disease diagnostics.[3]

Immunoassays are attractive for the rapid detection of *B. anthracis* spores, vegetative cells and toxins; however, the challenge of developing such assays is the identification of poly- or monoclonal antibodies that do not cross react with antigens of related species.[4] It has been recognized that the inherent binding specificity and lytic action of bacteriophage enzymes called endolysins offer an attractive alternative to antibodies for the detection of microbes.[5]

Bacteriophages, which are viruses that infect bacteria, have coevolved with their hosts to infect with high efficiency and specificity. During the final stage of infection, bacteriophages express enzymes known as endolysins, which cleave glycosidic or peptide bonds of peptidoglycan resulting in cell lysis and release of bacteriophages. Endolysins are usually chimaeric proteins composed of a well-conserved catalytic domain fused to a largely divergent cell wall binding domain that directs the enzyme to a substrate. In many cases, the cell wall binding domain recognizes polysaccharides that are essential for viability. Molecular mechanisms that confer high affinity and selectivity to these carbohydrate-binding domains are not understood.

B. anthracis lysins such PlyB, PlyG and PlyL can specifically lyse *B. anthracis in vitro* and *in vivo* and have attracted attention for the development of novel antimicrobial agents and early disease diagnostics.[6] PlyB is bactericidal when applied as a full-length protein, whereas the catalytic domain alone showed no detectable lytic activity.[7] On the other hand, the recombinant catalytic domain of PlyL shows lytic activity, however, it displays a much larger host range compared to the full-length lysin.[8] These and other findings highlight the importance of the carbohydrate domain for selective targeting of host bacteria.

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The carbohydrate binding domain of *B. anthracis* endolysins targets secondary cell wall polysaccharides.[9] The vegetative cell wall of bacteria *B. anthracis* is composed of a peptidoglycan layer, which underlies an exterior coating of surface-layer (S-layer) glycoproteins. It also contains a secondary cell wall polysaccharide that is attached to muramic acid of peptidoglycan through a phosphodiester linkage and is critically involved in the organization of the cell wall by anchoring S-layer proteins to the bacterial cell membrane through noncovalent interactions.[10,11] The polysaccharide is composed of a →6)-α-D-GlcNAc-(1→4)-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→) backbone that is branched at C-3 and C-4 of the α-D-GlcNAc moiety with α-D-Gal and β-D-Gal, respectively, and at C-3 of the β-GlcNAc residue with a α-Gal (Figure 1).[12,13] These positions are, however, only partially substituted resulting in considerable microheterogeneity.

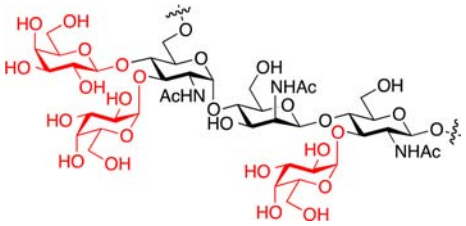

Figure 1. The structure of the secondary cell wall polysaccharide of *B. anthracis* vegetative cells.

To uncover molecular mechanisms by which *B. anthracis* lysins target secondary cell wall polysaccharides, compounds **1**−**7** (Figure 2) were chemically synthesized for binding studies with PlyL and PlyG using surface plasmon resonance (SPR). Compound **1** is a hexasaccharide composed of the trisaccharide backbone modified at all relevant positions with a galactosyl moiety. Compound **2** represents the backbone trisaccharide and tetrasaccharides **3**, **4**, and **5** have an additional galactosyl moiety at each of the possible branching positions. Compound **6** is the backbone trisaccharide lacking the terminal *N*-acetyl-glucosamine moiety and compound **7** is a derivative of trisaccharide **2** in which the terminal *N*-acetyl-glucosamine moiety is replaced by *N*-acetyl-galactosamine. This structure represents the backbone of the secondary cell wall polysaccharide of *Bacillus cereus*, which is closely related to *B. anthracis*.[14] The compounds could readily be prepared by employing a core trisaccharide modified by the orthogonal protecting groups N^{α}-9-fluorenylmethyloxycarbonate (Fmoc), 2-methylnaphthyl ether (Nap), levulinoyl ester (Lev) and dimethylthexylsilyl ether (TDS), which in a sequential manner could be removed to install side chain galactosides or modify the anomeric center. The binding studies demonstrated that PlyL and PlyG exhibit similar structure activity relationship for the synthetic compounds and exceptional high affinity binding was observed for monogalactosylated tetrasaccharide **4.** Furthermore, the pattern of galactosylation greatly influenced binding. A β-linked galactosyl moiety to C-4 of the non-reducing GlcNAc significantly increased affinity whereas an α-galactoside at C-3 of this residue greatly reduced binding. An α-galactoside at C-3 of GlcNAc at the reducing end can be accommodated by the PlyL and PlyG but does not significantly increase the binding affinity. It is proposed here that the pattern of galactosylation required for high affinity binding offers specificity for specific bacilli species.

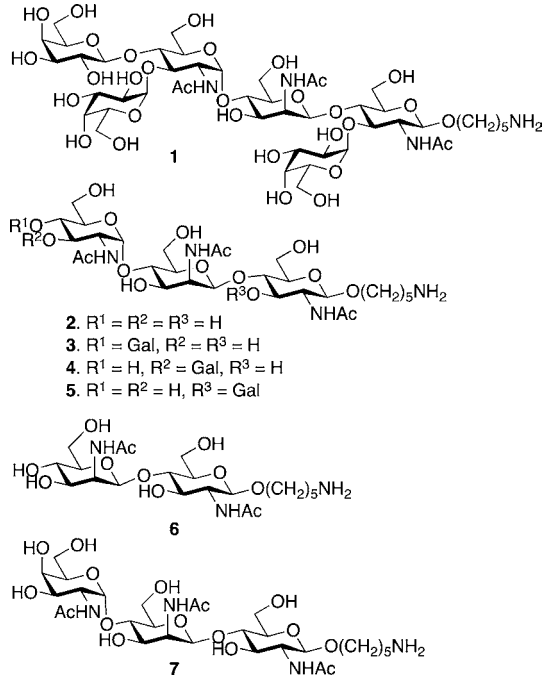

Figure 2. Synthetic targets for binding studies.

RESULTS

Chemical Synthesis. It was envisaged that compounds **1**−**5** could be prepared from common trisaccharides **8**, which at key positions is modified by the orthogonal protecting groups N^{α}-9-fluorenylmethyloxycarbonate (Fmoc),[15] 2-methylnaphthyl ether (Nap),[16] levulinoyl ester (Lev),[17] and dimethylthexylsilyl ether (TDS) (Figure 3). It was expected that the Fmoc carbamate could be selectively cleaved by treatment with a non-nucleophilic base such as triethylamine, whereas the Lev ester is susceptible to treatment with hydrazine acetate[18] and this reagent should not affect any of the other protecting groups. The Nap ether can be removed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and these conditions should be compatible with the other functions.[16] The anomeric center of **8** is protected as a dimethylthexylsilyl (TDS) ether, which can be selectively unmasked by treatment with hydrogen fluoride in pyridine. The resulting hemiacetal can then be converted into the trichloroacetimidate[19] for further glyco-

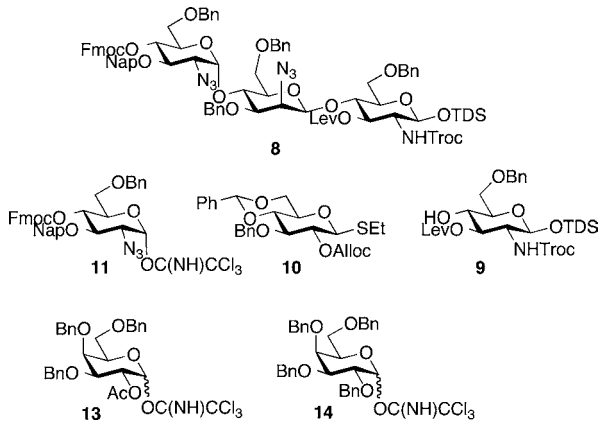

Figure 3. Building blocks for assembly of oligosaccharides.

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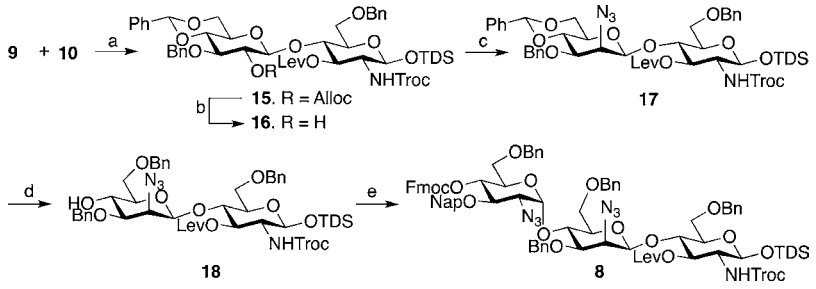
Scheme 1[a]



[a]Reagents and conditions: (a) NIS, TMSOTf, DCM, −20 °C (86%); (b) $Pd(PPh_3)_4$, THF/H_2O (91%) (c) Tf_2O, DMAP, Py/DCM, 0 °C followed by NaN_3, DMF, 55 °C (86%, 2 steps); (d) Et_3SiH, TfOH, DCM, −78 to −35 °C (65%); (e) **11**, TMSOTf, Et_2O/DCM, −55 to 0 °C (54%, α/β = 8/1).

sylation to provide selectively a β-anomer due to neighboring participating by the 2,2,2-trichloroethyoxycarbamate (Troc) function.[20] In this way, an anomeric *N*-benzyl-*N*-benzyloxycarbonyl pentanol moiety was introduced, which provides an opportunity for future conjugations.

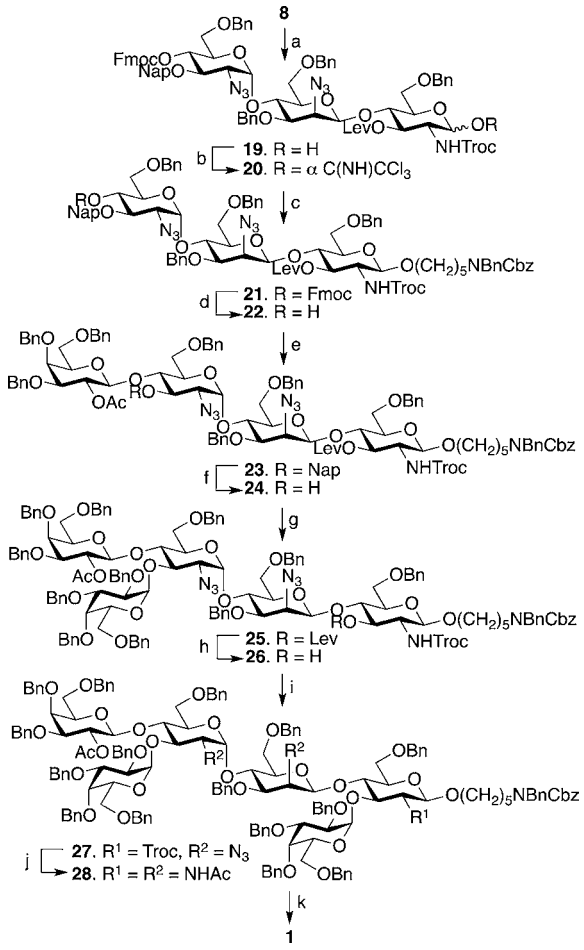
Sequential removal of the protecting groups of **8** and galactosylation of the resulting acceptors with galactosyl donors **13**[21] or **14**[22] should, after global deprotection, provide target hexasaccharide **1**. On the other hand, complete deprotection of **8** should give the backbone trisaccharide **2**, whereas selective removal of one of the orthogonal protection groups followed by the introduction of a single galactoside and deprotection should give access to tetrasaccharides **3**, **4**, and **5**.

Trisaccharide **8** was assembled from monosaccharide building blocks **9**, **10**, and **11**, which were readily be prepared by standard procedures (Figure 3). Thus, the challenging β-mannosamine moiety was introduced by a strategy whereby initially a β-glucoside is installed by coupling acceptor **10** with glucosyl donor **9** having a participating ester protecting group at C-2 to control β-anomeric selectivity.[23] Next, the C-2 Alloc group was removed and the resulting hydroxyl converted into a leaving group, which was then displaced by an azide to give a 2-azido-β-D-mannoside.

Thus, condensation of thioglucoside **10** with acceptor **9** using NIS and TMSOTf as the promoter[24] at −20 °C afforded disaccharide **15** in a yield of 86% as only the β-anomer (Scheme 1). The allyloxycarbonate moiety of **15** was removed by treatment with catalytic amount of $Pd(PPh_3)_4$ in a mixture of THF and water to provide **16** in a high yield. Compound **16** was treated with triflic anhydride (Tf_2O) in the presence of catalytic 4-dimethylaminopyridine (DMAP) in a mixture of pyridine and dichloromethane and the resulting triflate was displaced by sodium azide in DMF at 55 °C to afford azido-mannoside **17** in a yield of 86% over two steps. Selective reductive ring-opening of the benzylidene acetal of **17** was achieved using Et_3SiH and TfOH[25] to provide acceptor **18**, which was coupled with trichloroacetimidate **11** in a mixture of Et_2O/DCM to give trisaccharide **8** in a yield of 54% as mainly the α-anomer (α/β = 8/1).

Next, the anomeric aminopentyl linker was introduced by a three-step process to give **21** by selective removal of the anomeric TDS ether of **8** by treatment with hydrogen fluoride-pyridine in THF to give hemiacetal **19**, which was transformed into the corresponding trichloroacetimidate **20** by reaction with trichloroacetonitrile in the presence of K_2CO_3, and finally, a TMSOTf-catalyzed glycosylation of **20** with aminopentyl linker **12** (Scheme 2).

Scheme 2[a]



[a]Reagents and conditions: (a) HFPy, THF (92%); (b) Cl_3CCN, K_2CO_3, DCM (91%); (c) *N*-benzyl-*N*-benzyloxycarbonyl-**5**-amino-pentanol (**12**), TMSOTf, DCM, −30 to −20 °C (87%); (d) Et_3N/DCM (98%); (e) **13**, TMSOTf, DCM, −35 to −25 °C (88%); (f) DDQ, DCM/H_2O (78%); (g) **14**, TMSOTf, Et_2O/DCM, −55 to 0 °C (72%); (h) H_2NNH_2, HOAc, DCM/MeOH; (93%); (i) **14**, TMSOTf, Et_2O/DCM, −55 to 0 °C (65%, α/β > 20/1); (j) Zn, Ac_2O/AcOH/THF, $CuSO_{4(aq)}$ (72%); (k) NaOMe, MeOH/DCM followed by $Pd(OH)_2$/C, H_2, tBuOH/AcOH/H_2O (69%, 2 steps).

Removal of the Fmoc carbamate of **21** was accomplished by reaction with triethylamine in DCM without affecting the other protecting group to give the corresponding trisaccharide acceptor **22**. A TMSOTf catalyzed glycosylation of trichlor-

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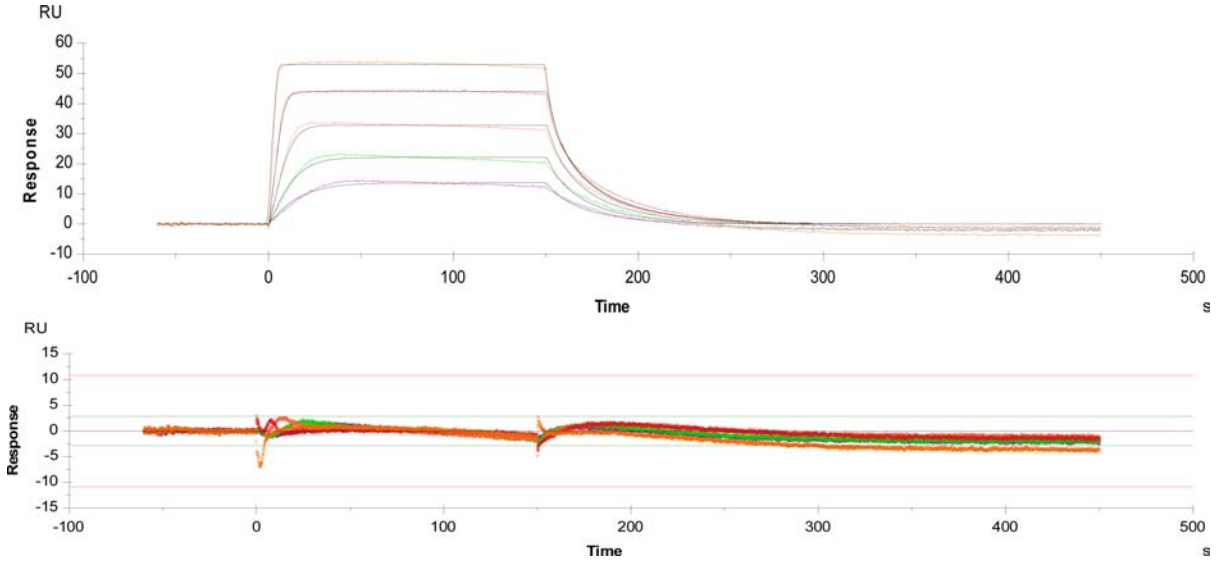

Figure 4. Sensorgram representing the concentration-dependent kinetic analysis of the binding of trisaccharide **2** with immobilized PlyL. It shows the simultaneous kinetic analysis of 2-fold serial dilutions of **2** at concentrations of 100 to 6.25 μM, fitted with a Langmuir 1:1 binding model (black lines).

oacetimidate **13**[19] with **22** afforded tetrasaccharide **23** in high yield as only the β-anomer. The Nap ether of **23** was removed oxidatively by reaction with DDQ[16] to give the corresponding tetrasaccharide acceptor **24**. As expected, the other two protecting groups remained intact. Coupling of acceptor **24** with trichloroacetimidate donor **14**[22] by using catalytic TMSOTf in a mixture of Et_2O/DCM provided pentasaccharide **25** in a yield of 72% as only the α anomer. Next, the Lev ester of **25** was removed by reaction with hydrazine acetate,[17,18] and the resulting pentasaccharide acceptor **26** coupled with **14** by employing TMSOTf in a mixture of Et_2O/DCM provided the desired hexasaccharide **27** as mainly the α anomer (65%, α/β > 20/1). Hexasaccharide **27** was treated with Zn in a solution of AcOH/Ac_2O/THF and aqueous $CuSO_4$[26] to convert the azido and Troc groups into acetimido moieties. Deacetylation of **28**, followed by hydrogenolysis over $Pd(OH)_2$/C in a mixture of ^{t}BuOH/AcOH/H_2O furnished the hexasaccharide **1** in a yield of 69% in two steps.

Trisaccharide **2** was prepared by standard global deprotection of **21**. Tetrasaccharides **3**, **4** and **5** were prepared by selective removal of Fmoc, Nap or Lev of **21** followed by galactosylation with **13** or **14** and global deprotection (see SI for details). Compounds **6** and **7** were assembled by a similar approach as used for **8** (see SI for details).

Binding Studies. SPR is a rapid and sensitive method for the evaluation of affinities of biomolecular interactions[27] that has as a benefit on relying exclusively on mass changes, thus allowing the study of interactions in real time without the need for external labels such as fluorophores, which in some cases can alter the nature of the interaction. Collecting SPR data for low molecular weight analytes such as compounds **1**–**7** is challenging because the refractive index monitored during a binding event is relatively small, and thus results in responses with much lower magnitudes than those observed in typical protein–protein interactions. In spite of these challenges, the high sensitivity and reproducibility of modern instruments combined with proper experimental design permits the direct monitoring of binding of low molecular weight analytes to immobilized proteins.[28]

The cell wall binding domains of PlyL and PlyG were immobilized on NHS-activated groups of a CM-5 research grade sensor chip surface and titration experiments were performed with the synthetic compounds **1**–**7**. Good agreements between the theoretically predicted and experimentally observed maximal specific binding (R_{max}) parameters indicated that amine immobilization of the protein yielded fully active surfaces. The high mass transport coefficients (>10^{10}) obtained from the kinetic model fit with mass transport limitation (MTL) experiments using the MTL wizard were indicative of insignificant mass transport effects and negligible rebinding of the analyte during the post injection phase (data not shown). For all SPR experiments, bulk refraction caused by the difference in the refractive index of the running buffer and sample injection and nonspecific binding were negated by using a control cell functionalized by ethanolamine.

A representative sensorgram for the interaction of trisaccharide **2** with immobilized PlyL is shown in Figure 4. A 1:1 Langmuir binding model gave a good fit (χ^2 = 1.26) and provided a K_D of 13.5 μM (Table 1). Interestingly,

Table 1. Dissociation Constants for the Binding of PlyL and PlyG Measured by SPR

analyte	K_D (μm) PlyL	K_D (μm) PlyG
1	23.0 ± 2.0	23.3 ± 0.5
2	13.5 ± 1.5	8.6 ± 0.4
3	1.1 ± 0.2	2.1 ± 1.1
4	153 ± 10	136.0 ± 7
5	6.2 ± 2.0	4.1 ± 0.5
6	110.0 ± 9.0	49 ± 2.0
7	43 ± 11	19.5 ± 7.5

tetrasaccharide **3**, which has an additional β-galactoside at C-4 of the terminal GlcNAc moiety, exhibited significantly stronger binding (K_D = 1.1 μM) indicating that the β-Gal moiety substantially contributes to binding. On the other hand, tetrasaccharide **4** complexed poorly with the protein and this result suggests that the α-galactoside at C-3 of the terminal GlcNAc moiety cannot be readily accommodated by PlyL.

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Tetrasaccharide **5** showed a similar dissociation constant as trisaccharide **2**, which suggests that the α-Gal moiety at the reducing GlcNAc moiety can be accommodated by the protein but does not contribute to binding. Finally, hexasaccharide **1** was found to bind with a 10-fold reduced affinity compared to tetrasaccahride **3**, which is in agreement with the observation that the α-Gal moiety at C-3 of the terminal GlcNAc residue of the core trisaccharide cannot be properly accommodated by the protein.

PlyG and PlyL exhibit similar affinities for the range of synthetic compounds (Table 1), and thus, it appears that the two endolysins have evolved in such a way that they recognize a saccharide epitope with similar galactosylation pattern. On the basis of the 68 C-terminal residues that constitute the cell-wall binding domain, PlyL and PlyG share 64% identity and 78% homology. The differences are mainly attributed to residues with substitution of side chains with very similar chemical properties. Therefore, the folds of these proteins are likely to be similar, and may provide similar binding properties for cell wall oligosaccharides. Further structural studies will be required to establish the molecular mechanisms that confer high affinity and selectivity for the glycans. Future studies should include larger synthetic oligosaccharides to further validate that both endolysins recognize a similar carbohydrate epitope as oligosaccharide length may modulate binding.

■ DISCUSSION

Although it is appreciated that cell wall binding domains of endolysins of bacteriophages confer host selectivity,[5] molecular mechanisms that can account for unique targeting are not understood. To address this deficiency, we have chemically synthesized a range of well-defined oligosaccharides derived from the secondary cell wall polysaccharides of *B. anthraces* and employed these compounds to dissect ligand requirements for the carbohydrate binding domain of the endolysins PlyL and PlyG using SPR.

Secondary cell wall polysaccharides of *B. anthracis* have been implicated as ligands for endolysins,[9] however, this polysaccharide is structurally heterogeneous and contains structural elements found in other bacillus species. In this respect, cell wall polysaccharides from various bacilli have both common and variable structural features.[11,13,29] They contain an oligosaccharide-repeating unit rich in aminoglycosyl residues and contain at least a residue having manno- and gluco-configuration. Furthermore, quantitative glycosyl analysis has showed that strains belonging to the same lineage differ in relatively quantities of various monosaccharides, indicating the presence of strain- specific cell wall carbohydrates. It has also been observed that *B. anthracis* strains cell wall glycosyl compositions differ from one another in a plasmid-dependent manner and in particular in the absence of the plasmid pXO2 the ManNAc/Gal ratio was significantly decreased.[13] The results reported here indicate that the carbohydrate binding domain of PlyL and PlyG have evolved in such a way that they require unique features of the trisaccharide backbone and galactosyl substitution pattern for high affinity binding and it is likely that these structural motifs confer selectivity for the host.

Another remarkably observation reported here is that a simple tetrasaccharide can bind with exceptional high affinity with the protein target. In this respect, complexation of glycan bind proteins with monovalent ligands is in general of low affinity and in the millimolar range. In biological systems, multivalent binding events in which multiple ligands on one entity simultaneously interact with multiple receptors on a complementary entity provide high avidity and selectivity.[30] Recently, several bacterial lectins have been described that display micromolar range affinities for their saccharide ligands.[31] In one case, high affinity was achieved by a unique involvement of two calcium ions in the binding site and displacement of water molecules complex to the metals by saccharide ligands resulted in a gain of entropy.[32] In other cases, the rigidity of branched oligosaccharides, such as the blood group antigens, appear to improve the binding entropy term.[33] Interestingly, PlyL, which does not contain metal ions, can complex a linear tetrasaccharide (**3**) with exceptional high affinity. Structural studies will be required to uncover the molecular features that account for the high affinity binding.

■ CONCLUSION

The high specificity and activity of endolysins makes them attractive for *in vitro* and *in vivo* applications in food science, microbial diagnostics, and for the treatment of infectious diseases.[5] They also provide potential tools for molecular biology, and biotechnology. These promises will only be realized if ligand requirements of endolysins can accurately be defined. This paper demonstrates, for the first time, that such information can be obtained when detailed knowledge is available of structures of cell wall polysaccharides, which can guide the chemically synthesis of part structures for binding studies. A potential stumbling block in such an approach is the difficulty of preparing libraries of well-defined oligosaccharides and previous efforts to prepare the secondary cell wall polysaccharide of *B. anthracis* had focused on the synthesis of one compound at the time.[34] Here we address this difficulty by preparing a backbone trisaccharide that at key branching positions is modified by orthogonal protecting groups.[18,35] This feature made it possible to prepare rapidly a library of structurally related compounds for structure−activity relationships. Specifically, we demonstrate here that the protecting groups N^{α}-9-fluorenylmethyloxycarbonate (Fmoc), 2-methylnaphthyl ether (Nap), levulinoyl ester (Lev) and dimethylthexylsilyl ether (TDS) are orthogonal and provide chemical flexibility. This set of protecting group should facilitate the preparation of many other biologically important oligosaccharides and glycoconjugates.

■ EXPERIMENTAL SECTION

Reagents and General Procedures. Reagents were obtained from commercial sources and used as purchase. Dichloromethane (DCM) was freshly distilled using standard procedures. Other organic solvents were purchased anhydrous and used without further purification. Unless otherwise noted, all reactions were carried out at room temperature in oven-dried glassware with magnetic stirring. Molecular sieves were flame-dried under high vacuum prior to use. Organic solutions were concentrated under diminished pressure with bath temperatures <40 °C. Flash column chromatography was carried out on silica gel G60 (Silicycle, 60−200 μm, 60 Å). Thin-layer chromatography (TLC) was carried out on Silica gel 60 F_{254} (EMD Chemicals, Inc.) with detection by UV absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ∼150 °C or by spraying with a solution of $(NH_4)_6Mo_7O_{24}.H_2O$ (25 g/L) in 10% sulfuric acid in ethanol followed by charring at ∼150 °C. ^1H and ^{13}C NMR spectra were recorded on a Varian Inova-300 (300/75 MHz), a Varian Inova-500 (500/125 MHz) and a Varian Inova-600 (600/150 MHz) spectrometer equipped with sun workstations. Multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). Spectra were assigned using COSY, DEPT and HSQC

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experiments. All chemical shifts are quoted on the δ-scale in parts per million (ppm). Residual solvent signals were used as an internal reference. Reverse-Phase HPLC was performed on an Aglient 1200 series system equipped with an autosampler, fraction-collector, UV-detector and eclipse XDB-C18 column (5 μm, 4.6 × 250 mm) at a flow rate of 1.5 mL/min. Mass spectra were recorded on an Applied Biosystems 5800 MALDI-TOF proteomics analyzer. The matrix used was 2,5-dihydroxybenzoic acid (DHB) and ultamark 1621 as the internal standard.

General Procedure for Reductive Opening of a 4,6-Benzylidene. A mixture of the starting material (30 mM solution) and 4 Å molecular sieves (1.5 times the weight of starting material) in DCM was stirred under an atmosphere of Ar for 1 h. The reaction was cooled (−78 °C) and triethylsilane (2.5 equiv) was added followed by the addition of triflic acid (2.2 equiv). After the reaction was kept at −78 °C or warmed to −40 °C over a period of time until TLC analysis (EtOAc/hexanes) indicated disappearance of starting material, it was quenched by the addition of MeOH. The mixture was filtered, and the filtrate was washed with saturated $NaHCO_3$, brine. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash chromatography over silica gel (EtOAc/hexanes) to give pure product.

General Procedure for Deprotection of Nap Ethers. To a stirred solution of starting material (10 mM solution) in a mixture of DCM/water (10/1, v/v) was added DDQ (3 equiv). The reaction mixture was vigorously stirred in dark until TLC analysis (EtOAc/hexanes) indicated disappearance of starting material (~4 h). Next, the reaction mixture was diluted with DCM and washed with saturated $NaHCO_3$ brine. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography over silica gel (EtOAc/hexanes) to give pure product.

General Procedure for Deprotection of Lev Esters. To a stirred solution of starting material (10 mM solution) in a mixture of MeOH/DCM (1/8, v/v) was added hydrazine acetate (2 equiv). The reaction mixture was stirred until TLC analysis (EtOAc/hexanes) indicated disappearance of starting material (2 h), after which it was diluted with DCM and washed with saturated $NaHCO_3$, brine. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography over silica gel (EtOAc/hexanes) to give pure product.

General Procedure of TMSOTf-Mediated Glycosylation for Synthesis of α-Anomers. A mixture of the acceptor (1 equiv) and trichloroacetimidate (2 to 3 equiv), 4 Å molecular sieves (1.5 times the combined weight of donor and acceptor) in a mixture Et_2O/DCM (5/1, v/v, reaction concentration at ~0.03 to 0.05 M) was stirred under an atmosphere of Ar for 1 h. The reaction was cooled (−55 °C) and TMSOTf (0.2 equiv) was added. After the reaction temperature was slowly increased to 0 °C over a period of 1.5−2 h, it was quenched by the addition of pyridine (0.2 mL). The mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography over silica gel (EtOAc/hexanes or acetone/toluene) to give pure product.

General Procedure of TMSOTf-Mediated Glycosylation for Synthesis of β-Anomers. A mixture of the acceptor (1 equiv) and trichloroacetimidate (0.33−2.5 equiv), 4 Å molecular sieves (1.5 times the combined weight of donor and acceptor) in DCM (reaction concentration at ~0.06 to 0.08 M) was stirred under an atmosphere of Ar for 1 h. The reaction was cooled (−30 °C) and TMSOTf (0.2 equiv) was added. After stirring at −25 °C for 1 h, the reaction was quenched by the addition of pyridine (0.2 mL). The mixture was filtered, and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography over silica gel (EtOAc/hexanes) to give pure product.

General Procedure for Reduction of Azido and Troc Groups. To a stirred solution of starting material (3 mM solution) in AcOH/Ac_2O/THF (1/2/3, v/v/v) was added metallic zinc power (50 equiv) followed by saturated $CuSO_4$(aq) (0.2 mL). After stirring until HR MALDI-TOF MS indicated disappearance of starting material (45 min.), the reaction mixture was filtered, and the filtrate was washed with saturated $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure. The resulting yellow oil was purified by flash chromatography over silica gel (MeOH/DCM) to give pure product.

General Procedure for Ester and Carbamate Deprotection. To a stirred solution of starting material (2.5 mM solution) in a mixture of MeOH/DCM (1/2, v/v) was added a methanolic NaOMe solution (10 equivalents, 1.0 M). The resulting reaction mixture was stirred for 12 h. after which it was neutralized with 10% AcOH in MeOH. The resulting solution was diluted with DCM and washed with saturated $NaHCO_3$ and brine. The organic phase was dried ($MgSO_4$), filtered and the filtrate was concentrated under reduced pressure to afford an amorphous white solid. The product used directly in next step.

General Procedure for Hydrogenolysis. To a stirred solution of starting material (~3 mM solution) in a mixture of ^{t}BuOH/AcOH/H_2O (40/1/1, v/v/v) and $Pd(OH)_2$/C (2 times the weight of starting material, 20 wt.%, Degussa type) was added. The resulting mixture was placed under a hydrogen atmosphere (1 psi). After stirring for 24 h, the catalyst was filtered off and washed thoroughly with MeOH. The combined filtrates were concentrated under reduced pressure. The resulting yellow solid was purified by reversed phase HPLC on an analytical C-18 column using a gradient of 0 → 100% acetonitrile in H_2O over a 25 min period to give, after lyophilization of the appropriate fractions, an amorphous white solid.

Cloning, Expression, and Purification of the PlyL and PlyG Cell Wall Binding Domain. The preparation of plyL CWB was performed according to a previously reported procedure. The gene of the PlyG C-terminus was codon-optimized for *Escherichia coli* and synthesized from amino acids Lys166 to Lys233 based on NCBI accession DQ221100 for the PlyG full-length protein. The resulting construct, with a methionine added in front of Lys166, was inserted into the vector pET15b (Novagen) between NdeI and BamHI restriction sites. BL21DE3 (Agilent) was used for expressing theproteins following standard protocols. The lysis buffer (20 mM Tris-Cl, 300 mM NaCl, and 0.5% Triton X-100, at pH 7.0) was used to resuspend the cell pellet. Cells were lysed by French-press with 3 passes at 1000 PSI and clarified by centrifugation at 18 000 rpm (Sorvall SS-34) for 1 h at 4 °C. The clarified lysate was loaded directly onto a HITRAP Ni-chelating column (GE Healthcare) and equilibrated with 50 mL of buffer A (20 mM Tris-Cl and 300 mM NaCl at pH 7.0). Unbound protein was washed out by buffer A containing imidazole (30 mM). The His-tagged protein was eluted by imidazole (0.3 M) in buffer A. Superdex S200 16/60 (GE Healthcare) gel filtration, equilibrated with PBS, was then used to further purify the target protein. The molecular weight of the final protein product was confirmed by MALDI-TOF/TOF.

SPR Measurements. Binding interactions between PlyL and PlyG and oligosaccahrides **1**−**7** were examined using a Biacore T100 biosensor system (Biacore Inc., GE Healthcare). The protein was immobilized by standard amine coupling using an amine coupling kit (Biacore Inc., GE Healthcare). The surface was activated using freshly mixed *N*-hydroxysuccimide (NHS; 100 mM) and 1-(3-dimethylaminopropyl)-ethylcarbodiimide (EDC; 391 mM) (1/1, v/v) in water. Next, PlyL or PlyG (50 μg/mL) in aqueous NaOAc (10 mM, pH 5.5) was passed over the chip surface until a ligand density of approximately 2500 RU was achieved. The remaining active esters were quenched by aqueous ethanolamine (1.0 M; pH 8.5). The control flow cell was activated with NHS and EDC followed by immediate quenching with ethanolamine. HBS-EP (0.01 M HEPES, 150 mM NaCl, 3 mM EDTA, 0.005% polysorbate 20; pH 7.4) and HBS-N (0.01 M HEPES, 150 mM NaCl; pH 7.4) were used as the running buffer for the immobilization and kinetic studies, respectively. Analytes were dissolved in running buffer and a flow rate of 30 μL/min was employed for association and dissociation at a constant temperature of 25 °C. A sequential 60 s injection of aqueous glycine-HCl (10 mM; pH 2.0) and 30 s injection of NaCl (1.5 M) at a flow rate of 50 μL/min were used for regeneration and achieved prior baseline status. With the use of the Biacore T100 evaluation software, the response

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curves of various analyte concentrations were globally fitted to a one to one or the two-state binding model.

■ ASSOCIATED CONTENT

Supporting Information

The preparation of the starting materials, assembly of the oligosaccharides and copies of NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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