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4,6-*O*-Pyruvyl Ketal Modified *N*-Acetylmannosamine of the Secondary Cell Wall Polysaccharide of *Bacillus anthracis* Is the Anchoring Residue for Its Surface Layer Proteins

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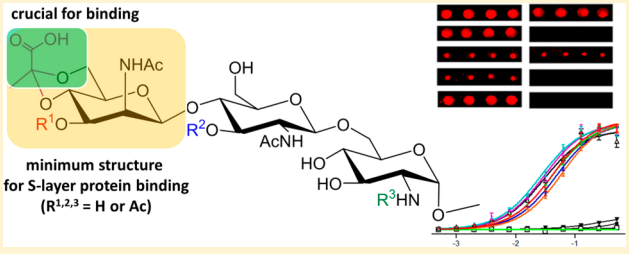
Supporting Information

ABSTRACT: The secondary cell wall polysaccharide (SCWP) of *Bacillus anthracis* plays a key role in the organization of the cell envelope of vegetative cells and is intimately involved in host−guest interactions. Genetic studies have indicated that it anchors S-layer and S-layer-associated proteins, which are involved in multiple vital biological functions, to the cell surface of *B. anthracis*. Phenotypic observations indicate that specific functional groups of the terminal unit of SCWP, including 4,6-*O*-pyruvyl ketal and acetyl esters, are important for binding of these proteins. These observations are based on genetic manipulations and have not been corroborated by direct binding studies. To address this issue, a synthetic strategy was developed that could provide a range of pyruvylated oligosaccharides derived from *B. anthracis* SCWP bearing base-labile acetyl esters and free amino groups. The resulting oligosaccharides were used in binding studies with a panel of S-layer and S-layer-associated proteins, which identified structural features of SCWP important for binding. A single pyruvylated ManNAc monosaccharide exhibited strong binding to all proteins, making it a promising structure for S-layer protein manipulation. The acetyl esters and free amine of SCWP did not significantly impact binding, and this observation is contrary to a proposed model in which SCWP acetylation is a prerequisite for association of some but not all S-layer and S-layer-associated proteins.



INTRODUCTION

S-layers are bidimensional para-crystalline protein surface layers that exist in nearly every taxonomic group of bacteria and represent an almost universal feature of Archaea.[1] The S-layer functions as a protective barrier with regularly spaced pores for selective permeability.[2] It is important for many biological functions such as maintenance of cell integrity, enzyme display, protection to phagocytosis, and interactions with the host and its immune system. Due to their critical functions and unique crystallization ability, S-layer proteins are of great interest for drug delivery, biomaterial engineering, and vaccine development.[3] Exploiting the biomedical potential of S-layer proteins will require a detailed understanding of how these proteins are tethered to the cell surface and assemble into stable two-dimensional surface layers.

The S-layer of *B. anthracis*, which is the causative agent of anthrax in humans and other mammals,[4] is mainly composed of surface array protein (Sap) and extractable antigen 1 (EA1).[5] These proteins are composed of an N-terminal S-layer homology (SLH) and a C-terminal crystallization domain.[5b] While the crystallization domain is responsible for the formation of a stable two-dimensional surface layer, the conserved SLH domain tethers the proteins to the cell surface.[6] The S-layer can contain up to 22 additional *B. anthracis* S-layer associated proteins (Bsl) as minor constituents.[2c] These proteins also possess an SLH domain for binding to the cell surface and an additional domain that exerts specific functions. For example, BslK is expressed under conditions of iron limitation and acts to scavenge heme for iron utilization enabling survival in the host. BslA facilitates infection by mediating adherence to host cells, thereby functioning as a virulence factor,[2c,7] and BslO has peptidoglycan hydrolase activity and plays a role in the separation of bacilli from elongating chains.[8] BslR, BslS, BslT, and BslU are predicted murein hydrolases.[9]

Previous biochemical studies have shown that the SLH domains of the S-layer and S-layer-associated proteins of *B. anthracis* are anchored to a cell wall associated polymer through noncovalent interactions.[10] Subsequent genetic studies demonstrated that *csaB*, which is the gene responsible for pyruvylation, is critical for retention of SLH-containing proteins.[11] Structural examinations by Carlson and co-workers revealed that the secondary cell wall polysaccharide (SCWP)

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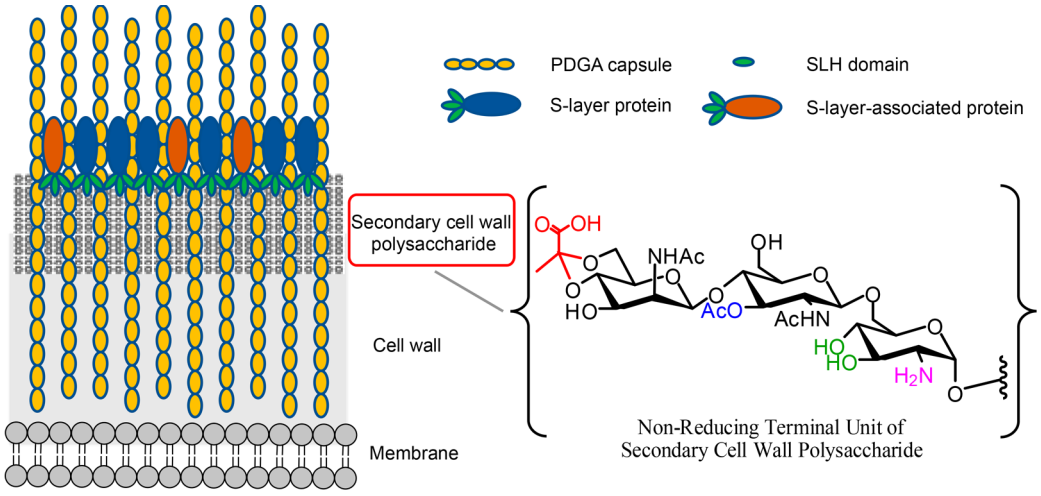

Figure 1. Schematic representation of the cell wall of *B. anthracis*. The cytoplasmic membrane of *B. anthracis* is encased in a thick layer of cell wall peptidoglycan that serves as the anchoring point for the secondary cell wall polysaccharide (SCWP) and the poly-γ-D-glutamate (PDGA) capsule. It is postulated that the trimeric SLH domain of S-layer and S-layer associated proteins are attached to SCWP through noncovalent binding.

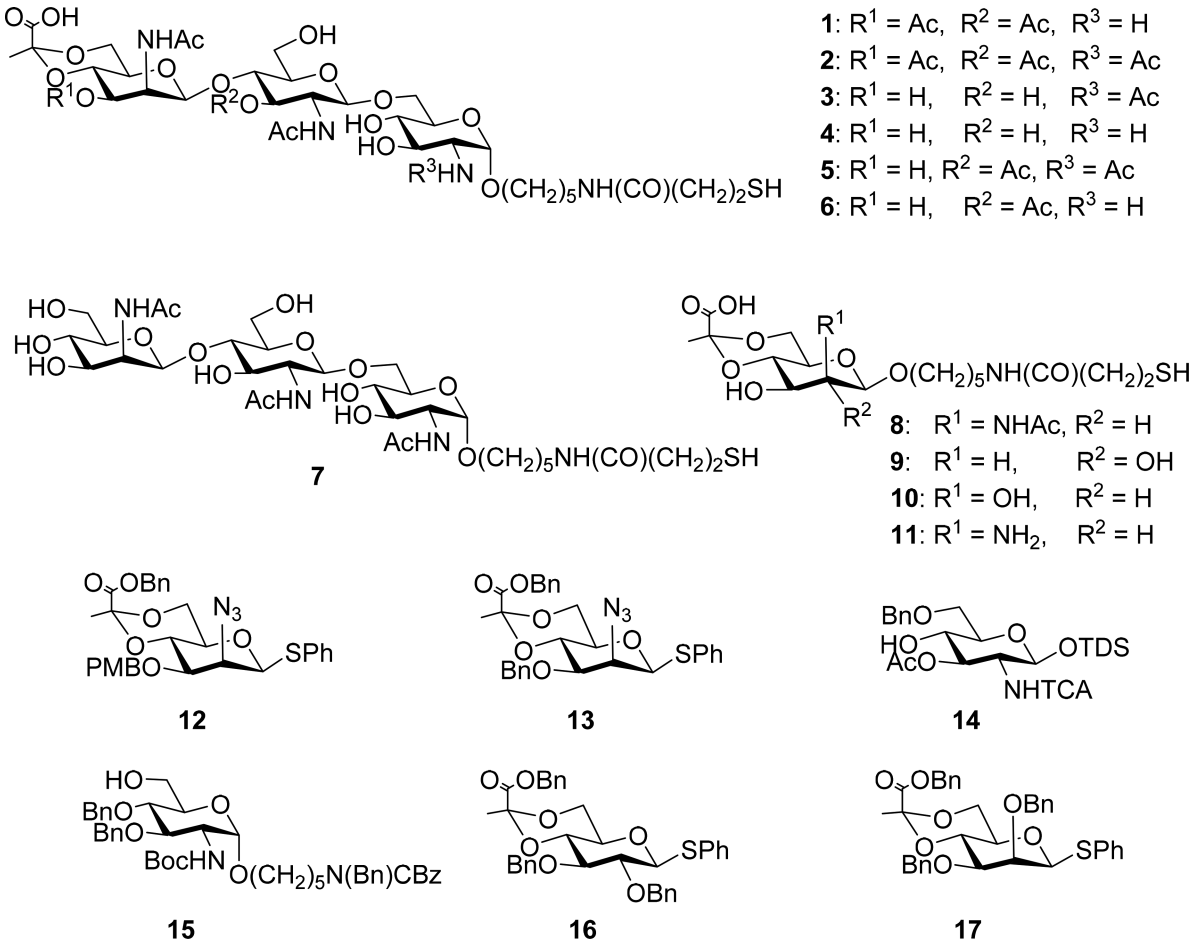

Figure 2. Synthetic targets for binding studies and building blocks for synthesis of *B. anthracis* SCWP derivatives.

of *B. anthracis* is modified by a pyruvyl ketal,[12] and it was postulated that this polysaccharide serves as the anchor for the SLH-containing proteins (Figure 1).

SCWP of *B. anthracis* is composed of trisaccharide repeating units having a structure of →4)-β-D-ManNAc-(1→4)-β-D-GlcNAc-(1→6)-α-D-GlcNAc-(1→ with nonstoichiometric galactosylation creating considerable heterogeneity (Figure 1). The distal trisaccharide is modified by a (*S*)-4,6-*O*-pyruvyl ketal on the β-ManNAc residue, and thus, this moiety is the putative ligand for SLH-containing proteins. The C-3 hydroxyl of the β-GlcNAc moiety of the distal repeating unit is nonstoichiometrically modified by an acetyl ester, and the α-GalNAc residue can be *N*-deacetylated (Figure 1).[13] A model for *O*-acetylation of SCWP has been proposed in which the

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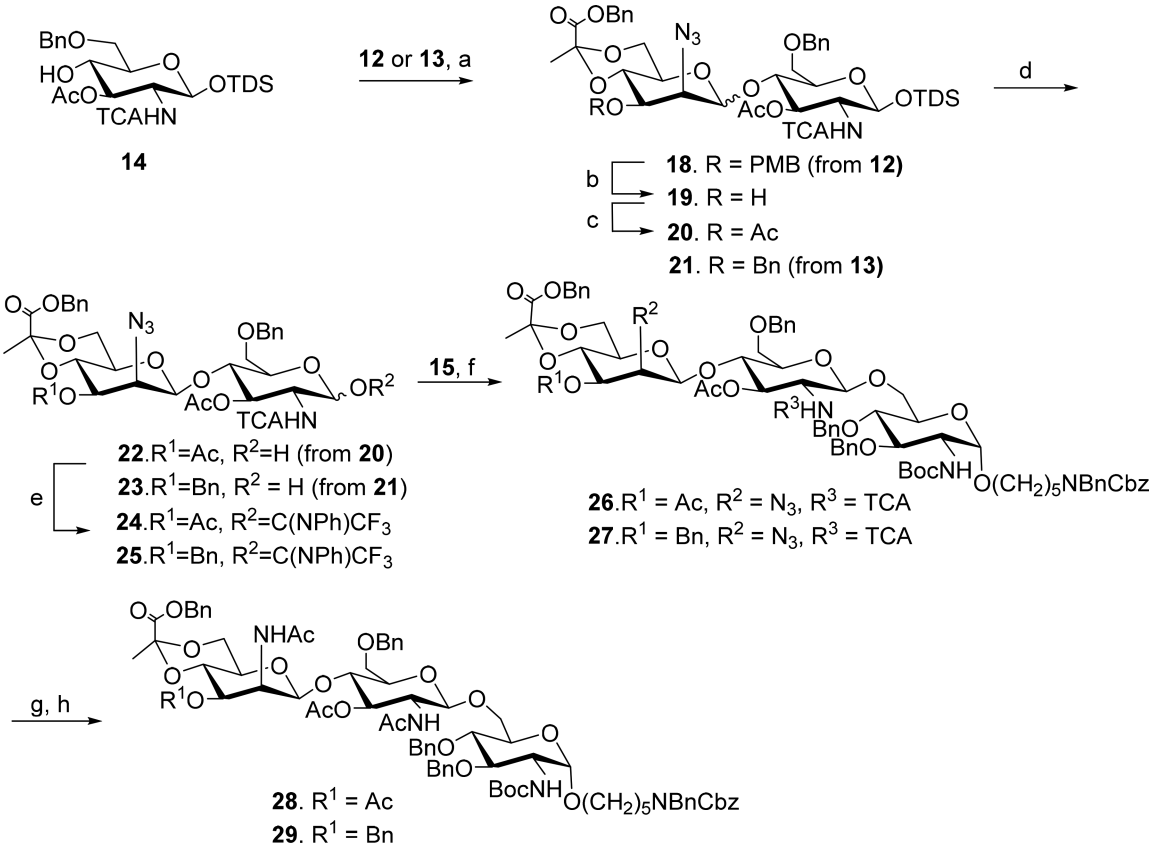
Scheme 1. General Synthetic Strategy[a]



[a]Reaction conditions: (a) (i) DPS, TTBP, Tf_2O, DCM, −78 to −55 °C; (ii) add acceptor dropwise, warm to rt (**18**: 71%, α/β = 1/1.2) (**21**: 74%, α/β = 1/1.1); (b) DDQ, DCM, PBS buffer pH 7.4, 84%; (c) Ac_2O/pyr, 100%; (d) HF-Py, THF (**22**: 90%; **23**: 88%); (e) $CF_3C(NPh)Cl$, Cs_2CO_3, DCM (**24**: 78%; **25**: 82%); (f) **15**, TMSOTf, DCM, −20 to 0 °C (**26**: 67%; **27**: 56%); (g) AIBN, Bu_3SnH, toluene; (h) Ac_2O/pyr (**28**: 96%, **29**: 75%, two steps).

membrane-bound *O*-acetyltransferases *patB1* performs the acetylation, whereas *patB2* is required for translocation of an acetyl donor across the plasma membrane from a cytoplasmic source.[14]

Genetic studies have indicated that fine structural details of SWCP are critical for proper S-layer formation.[11,15] In this respect, deletion of *patA1* and *patA2*, which significantly reduces *O*-acetylation of SWCP, leads to elongated chains of vegetative cells. Although the *patA1*/*patA2* mutant assembles an S-layer, it cannot retain the S-layer protein EA1 and is mainly composed of Sap. Furthermore, the deposition of the BslO, which is a murein hydrolase implicated in cell separation, was perturbed at the cell division septa. Compared to wild-type cells, the mutant displayed an aberrant deposition pattern of BslO, only covering half of the cell division septa. It was also observed that EA1, BslO, and BslA were not retained at the cell surface and released in the medium. The mutant produced quantities of SCWP comparable to wild-type cells but having substantially fewer acetyl esters. On the basis of these observations, it was proposed that *patA1*- and *patA2*-mediated *O*-acetylation of SCWP is important for retaining and depositing EA1, BslO, and BslA to specific regions of the cell envelope, which in turn is critical for proper cell division.[15]

The involvement of SCWP in anchoring S-layer and S-layer-associated proteins to cell surface is mainly based on genetic studies and has not been validated by detailed binding assays. Furthermore, the mechanism by which *O*-acetylation regulates the deposition of specific S-layer and S-layer associated proteins is not well understood. In large measure, this is due to the structural heterogeneity of SCWP, which complicates binding studies. Chemical synthesis is an attractive way to overcome this hurdle and in principle can provide well-defined structures for probing the binding between SCWP and proteins.

Herein, we report the chemical synthesis of seven trisaccharides and four monosaccharides derived from the distal unit of *B. anthracis* SCWP (Figure 2) that have perturbations in the 4,6-*O*-pyruvyl ketal and *O*- and *N*-acetylation patterns. The synthetic compounds were employed in microarray and ELISA binding assays to establish the importance of functional groups, such as the pyruvate ketal and the *O*- and *N*-acetyl groups, for binding. These studies confirm that SCWP functions as the anchoring point of the S-layer and S-layer-associated proteins, and it was found that only the terminal ManNAc moiety modified by a 4,6-ketal pyruvyl is important for binding. *O*-Acetylation of the GlcNAc moiety did, however, not substantially impact association with the various proteins, and this observation is contrary to a proposed model[15] in which SCWP acetylation is a prerequisite for the direct association of some but not all S-layer and S-layer-associated proteins. The role of *O*-acetylation of SCWP in proper S-layer formation is more complex than previously thought.

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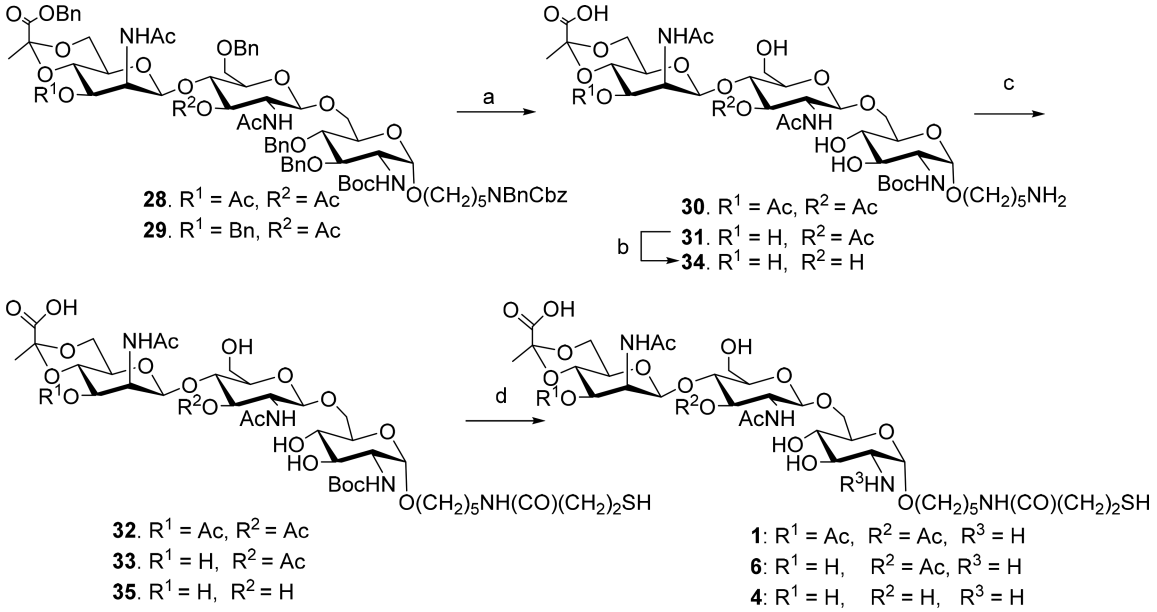
Scheme 2. General Synthetic Strategy[a]



[a]Reaction conditions: (a) $Pd(OH)_2$, *t*-BuOH, H_2O, H_2 (1 atm) (**30**: 67%; **31**: 57%); (b) LiOH, THF, H_2O (**34**: 73% from **31**); (c) (i) DTSSP, H_2O, pH ∼ 8; (ii) DTT, PBS buffer (pH 7.4), (**32**: 73% from **30**; **33**: 91% from **31**; **34**: 99% from **34**; (d) (i) 1/3 TFA/DCM, triethylsilane, 0 °C; (ii) DTT, PBS buffer (pH 7.4), (**1**: 71% from **32**; **6**: 83% from **33**; **4**: 83% from **35**).

RESULTS AND DISCUSSION

Chemical Synthesis. The trisaccharides **1**−**6**, which all contain a 4,6-*O*-pyruvyl ketal but differ in the pattern of *O*-acetylation and *N*-deacetylation, were prepared to probe the importance of these functionalities for S-layer protein binding (Figure 2). Trisaccharide **7**, lacking the pyruvyl ketal group, was included as a negative control because genetic studies had indicated that this functionality is critical for binding of S-layer-associated proteins.[11,16] The monosaccharides **8**−**11** were prepared to establish the minimum epitope and the involvement of the C-2 acetamido function of ManNAc for binding. The target compounds are modified by an aminopentyl linker for controlled coupling to a surface or carrier protein to facilitate binding studies.

The chemical synthesis of the trisaccharides represented a considerable challenge because it required accommodation of an acetyl ester, a pyruvyl ketal, and a free amine. To preserve the acetyl ester, base-cleavable protecting groups needed to be avoided during the deprotection steps. In addition, the presence of a carboxylic acid and free amine in the final products complicated the selection of a linker for conjugation purposes, and commonly employed linkers bearing an amino or carboxylic acid group could not be used. Therefore, a thiol-modified linker was selected, which was installed at a late stage of synthesis to avoid problems with poisoning the Pd catalyst required for the removal of benzyl ethers, which were selected for permanent protection. Furthermore, suitable C-2 amino protecting groups needed to be identified for selective amino group modification and for controlling stereoselective outcomes of glycosylations. The latter includes a β-linked 2-aminomannoside that is challenging to install due to unfavorable steric and electronic effects.[17] The poor reactivity of pyruvylated donors[18] further complicated the preparation of the target compounds.

A synthetic strategy was designed employing building blocks **12**−**17** that are modified by a carefully selected set of protecting groups (Figure 2). The pyruvyl ketal is protected as a benzyl (Bn) ester, which can be removed during the final hydrogenation step. A combination of azido (N_3), trichloroacetyl (TCA), *tert*-butoxycarbonyl (Boc), and benzyloxycarbonyl (Cbz) were utilized as amino protecting groups,[19] and their differential reactivity made it possible to selectively modify the various amino groups of the target compounds. Furthermore, TCA at C-2 of **14** can perform neighboring group participation during glycosylations to give selectively 1,2-*trans*-glycosides. Subsequently, TCA can be reduced to the desired acetamido function by treatment with Bu_3SnH and AIBN.[20] The Cbz protecting groups can be removed by catalytic hydrogenation in the presence of Boc, allowing selective installation of a thiol spacer on the amine of the linker. Next, Boc can be cleaved by using TFA/DCM, revealing the free amine of the target compounds. Building block **12** has a 3-*O*-*p*-methoxybenzyl (PMB) ether, which can be selectively removed to install an acetyl ester, while the 3-*O*-benzyl ether of **13** can be carried through the final stages of the synthesis to afford targets having a C3 hydroxyl.

Installation of the β-mannosamine linkage of the target trisaccharides proved to be challenging. It is well-known that preactivation of a mannosyl donor protected with a 4,6-*O*-benzylidene acetal provides mainly β-mannosides.[21] In this glycosylation, preactivation results in the formation of an α-triflate which in an S_N2 fashion is displaced by a sugar alcohol to give a β-mannoside. Glycosylation through direct displacement is favored due to conformational constraints and dipoles imposed by the benzylidene acetal.[22] This methodology has been successfully extended to the preparation of 2-azido-2-deoxy-β-mannosides.[23] Therefore, we were compelled to investigate whether the 4,6-*O*-pyruvyl ketal of glycosyl donors such as **12** and **13** can direct glycosylations to provide the required β-mannosyl product (Scheme 1). Thus, treatment of these glycosyl donors with diphenyl sulfoxide (DPS), trifluoromethanosulfonic anhydride (Tf_2O), and 2,4,6-tri-*tert*-

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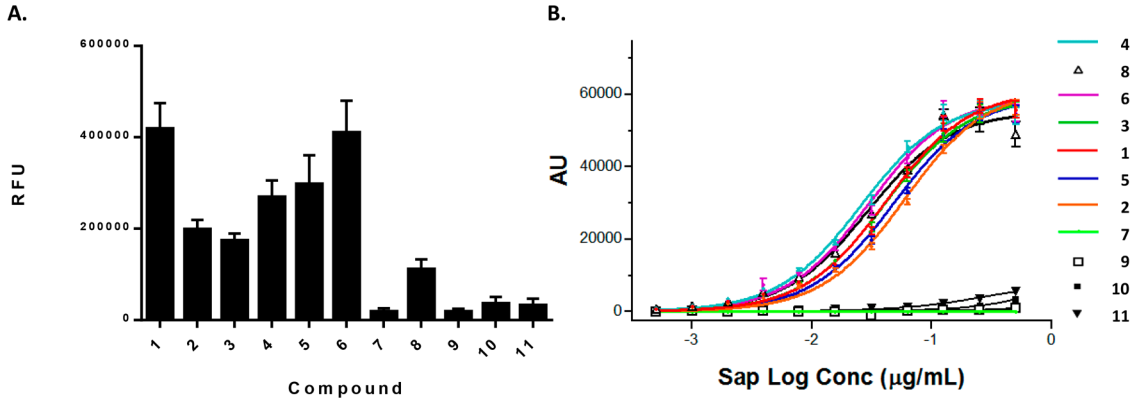

Figure 3. Microarray and ELISA results for the binding of Sap with compounds **1**−**11**. (A) Compounds **1**−**11** were printed onto maleimide-functionalized glass slides by piezoelectric microarray printing. A fusion protein of the SLH domain of Sap with mCherry (0.5 μg/mL) was screened for binding using an anti-mCherry antibody followed by addition of a fluorescently labeled secondary antibody for detection. (B) Dose−response curves for Sap determined by ELISA. Errors displayed as 95% confidence intervals.

butyl pyrimidine (TTBP) to form in situ a triflate was followed by the addition of acceptor **14** resulting in the formation of disaccharides **18** (71%) and **21** (74%), respectively (Scheme 1), as separable mixtures of α/β anomers (α/β = 1/1.2 and 1/1.1, respectively). These results indicate that a 4,6-*O*-pyruvyl ketal does not exert a β-directing effect and probably its axial benzyl ester induces an unfavorable dipole that hinders direct displace of the α-triflate. The ether protecting group at C-3 of donors **12** and **13** was important for the anomeric outcome of the glycosylations because the use of a similar donor having an acetyl ester at this position gave only α-anomeric product. Similar effects on stereochemical outcomes have been reported when using standard benzylidene-protected thioglycosides bearing a C-3 ester.[24]

We also explored the installation of the 1,2-*cis*-mannoside using a pyruvylated glucosyl donor having a participating ester at C-2. Such a donor makes it possible to install a 1,2-*trans* glucoside by exploiting neighboring group participation during glycosylation. Next, the C-2 ester can be removed and the resulting 2-hydroxyl converted into a leaving group for S_N2 displacement with azide, resulting in the formation of a β-linked 2-azidomannoside.[25] Several pyruvylated glucosyl donors and glycosylation conditions were examined, but in each case a low yield of disaccharide was obtained probably due to the low reactivity of the donors (see Scheme S2). Another strategy was attempted that involved installation of the pyruvyl moiety at the disaccharide stage.[26] Unfortunately, this strategy resulted in an inseparable mixture of diastereomers (see Scheme S5 for details).

Next, disaccharide **18** was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to remove the PMB ether[27] to give **19** that was acetylated with Ac_2O in pyridine. The resulting disaccharide **20** was converted into glycosyl donor **24** by first removing the anomeric TDS ether using an HF−pyridine complex followed by reaction of the lactol **22** with *N*-phenyltrifluoroacetimidoyl chloride in the presence of cesium carbonate. A TMSOTf-catalyzed glycosylation of **24** with **15** yielded trisaccharide **26** as the only β-glucoside due to neighboring group participation of the TCA function. Trisaccharide **27**, bearing a benzyl ether at the 3-*O* position of ManNAc, was prepared by a similar route starting from disaccharide **21**.

The TCA and azido moieties of **26** and **27** needed conversion into acetamido functions. The presence of the acid-sensitive Boc group precluded the use of Zn/acetic acid, which is commonly used for such transformations. Instead, a radical reduction of **26** and **27** using AIBN and Bu_3SnH was performed followed by acetylation with Ac_2O to provide the key trisaccharide intermediates **28** and **29** in excellent yield.

Deprotection of trisaccharides **28** and **29** with specific reagents provided target compounds **1**−**6** (Scheme 2). Thus, catalytic hydrogenation of **28** and **29** with $Pd(OH)_2$ resulted in the removal of the benzyl and benzyloxycarbonyl protecting groups. The free amine of the pentyl linker on the resulting compounds **30** and **31** was reacted with 3,3′-dithiobis(sulfosuccinimidyl propionate) (DTSSP) and then DTT to install a thiol moiety for conjugation purposes.[28] Removal of the Boc protecting group on **32** and **33** with TFA in DCM at 0 °C gave targets **1** and **6**, respectively. Compound **4** was obtained by first treating **31** with LiOH to remove the acetyl ester, followed by modification of the amine of the linker of **34** with DTSSP and DTT. Finally, cleavage of Boc on **35** using TFA/DCM at 0 °C gave target **4**.

Target compounds **2**, **3**, and **5** having an acetamido group were prepared by first treating **28** and **29** with TFA/DCM at 0 °C followed acetylation with acetic anhydride to afford the acetamido-modified trisaccharides. The compounds were carried forward in a manner similar to that illustrated in Scheme 2 to afford compounds **2**, **3**, and **5** (Schemes S11 and S12). The synthesis of the truncated targets employed a similar methodology (Schemes S13−S16). The nonpyruvylated trisaccharide (**7**) was synthesized as previously described.[14]

Binding Studies. The S layer of *B. anthracis* is composed of two S-layer proteins (Sap and EA1)[5] and can be further decorated with up to 22 S-layer associated proteins.[2c] The S-layer and S-layer-associated proteins contain an SLH domain that tethers the proteins to the cell surface via noncovalent binding to SCWP.[16,29] Genetic studies have indicated that pyruvylation of SCWP is essential for SLH binding.[11] Furthermore, *O*-acetylation of SCWP appears to be important for deposition of some but not all S-layer and S-layer-associated proteins and may play a role in positioning proteins at specific regions of the cell envelope.[15]

To establish the importance of specific structural features of SCWP for interaction with various S-layer and S-layer-associated proteins, the binding of the synthetic compounds **1**−**11** with recombinant SLH domains of Sap, EA1, BslA, BSlI, BslK, BslM, BslO, BslP, BslR, BslS, BslT, and BslU fused to

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Table 1. EC_{50} Values (μg/mL) of Compounds 1−11 Binding with the SLH Domains of the Various Proteins

compd	Sap	EA1	BslP	BslA	BslI	BslS	BslT	BslU	BslK	BslO	BslR	BslM
1	0.042	0.015	0.061	0.005	0.020	0.025	0.028	0.013	0.035	0.029	0.037	0.033
2	0.059	0.021	0.101	0.007	0.026	0.035	0.034	0.017	0.048	0.039	0.060	0.048
3	0.040	0.016	0.101	0.006	0.023	0.050	0.027	0.017	0.040	0.023	0.039	0.043
4	0.026	0.016	0.045	0.004	0.016	0.043	0.023	0.012	0.028	0.022	0.023	0.026
5	0.048	0.020	0.077	0.006	0.024	0.032	0.030	0.017	0.044	0.037	0.054	0.040
6	0.030	0.013	0.041	0.004	0.015	0.021	0.022	0.011	0.028	0.023	0.027	0.024
7	NB[a]	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
8	0.029	0.015	0.063	0.006	0.025	0.067	0.036	0.013	0.032	0.021	0.031	0.035
9	NB	NB	0.116	0.022	0.076	NB	NB	0.063	0.077	NB	>0.166	0.075
10	NB	NB	0.121	0.015	0.066	NB	NB	0.112	>0.177	NB	>0.192	0.066
11	NB	NB	0.036	0.006	0.027	NB	NB	0.077	>0.132	>0.111	0.073	0.052

[a]NB = no binding.

mCherry[16] was investigated. To rapidly provide qualitative binding information, a microarray screening assay was developed.[30] Thus, compounds **1**−**11**, having a thiol-containing linker, were immobilized onto maleimide-functionalized glass slides by piezoelectric microarray printing. The 12 mCherry fusion proteins were screened for binding using an anti-mCherry antibody followed by addition of secondary antibody modified by a fluorescent dye for detection. This approach was required because mCherry was not sufficiently fluorescent to allow direct detection of binding.

Figure 3A shows a representative microarray result for Sap. Trisaccharides **1**−**6**, bearing different patterns of acetyl esters and free amino groups, gave similar responsiveness. Compound **7**, lacking the 4,6-*O*-pyruvyl ketal, did not exhibit binding, which is in agreement with genetic studies that had indicated that this functionality is critical for S-layer formation.[11,31] Monosaccharide **8**, having a 4,6-*O*-pyruvyl ketal on ManNAc, showed binding to Sap, indicating that the terminal monosaccharide of CSWP is the key epitope for binding. The latter was supported by a lack of binding of monosaccharides **9** and **10** that have perturbations in the C-2 acetamido moiety. Microarray screening for binding for the other proteins indicated subtle differences in ligand requirements of the examined proteins (see Figure S1).

Next, binding affinities of the synthetic compounds with the various SLH domains were determined by ELISA (Figures 3B and S2). Thus, the thiol-containing oligosaccharides were conjugated to BSA modified by maleimide, and the resulting neoglycoconjugates were used for coating microtiter plates. Different concentrations of the various SLH domains were exposed to the coated microtiter plates to determine EC_{50} values. As can be seen in Table 1, all trisaccharides bearing a 4,6-*O*-pyruvyl ketal (compounds **1**−**6**) were recognized by the panel of proteins. *O*-Acetylation of β-GlcNAc (**1** vs **4**, **2** vs **5**) or ManNAc (**3** vs **5**, **4** vs **6**) had no or only a small effect on binding. In a number of cases, *O*-acetylation of β-GlcNAc led to a slight decrease in binding (**1** vs **4**, Sap and BslP). Furthermore, the free amine bearing compounds **1**, **4**, and **6** did not show a substantial difference in binding compared to their acetamido-containing counterparts **2**, **3**, and **5**, respectively.

The binding study supports that the terminal ManNAc moiety of SCWP modified by a 4,6-ketal pyruvyl ketal functions as the anchoring point of S-layer and S-layer-associated proteins.[11] It indicates that the role of *O*-acetylation of SCWP in S-layer formation is more complex than previously thought[15] and does not support a model in which SCWP acetylation is a prerequisite for the direct association of some (EA1, BslO, and BslA) but not of other S-layer and S-layer-associated proteins. Alternative models are therefore more probable, and *O*-acetylation of SCWP may for example be important for the proper localization of other proteins involved in S-layer formation such as the translocases SecA2 and SlaP that have been implicated in the transport of specific S-layer proteins to the cell surface.[32] The binding studies have been performed with individual proteins and does not probe possible retention of proteins through protein−protein complexes. In this respect, it may be possible that deposition of one protein that requires *O*-acetylated SCWP mediates the retention of other proteins.

Another interesting finding is that the terminal ManNAc moiety modified by a pyruvyl ketal is the minimal epitope for binding of all examined S-layer and S-layer associated proteins and monosaccharide **8** exhibited similar bindings (EC_{50} values) compared to the trisaccharide counterparts. However, we observed differences in the requirement of the axial acetamido moiety (monosaccharides **8** vs **9** and **10** vs **11**) which is critical for Sap, EA1, BslS, and BslT but not for BslP, BslA, BslL, BslU, BslK, BslR, and BslM, indicating that the SLH domains of various proteins utilize different structural features for binding, and further studies[33] are required to confirm such a mode of binding.

CONCLUSIONS

While S-layer and S-layer-associated proteins of *B. anthracis* play key roles in many important biological processes, mechanisms by which these proteins are deposited at the cell envelope to perform their functions are not well understood. We have designed and prepared a range of oligosaccharides derived from the terminal repeating unit of SCWP to examine whether specific functional groups, including 4,6-*O*-pyruvyl ketal and acetyl esters, can regulate the deposition of specific S-layer and S-layer-associated proteins. We developed a synthetic strategy that addresses difficulties related to the synthesis of pyruvylated oligosaccharides derived from *B. anthracis* SCWP bearing base-labile esters and free amino groups. Binding studies of a wide range of S-layer and S-layer-associated proteins with the resulting synthetic compounds provided direct biophysical evidence that SCWP functions as the anchoring point of these proteins and confirmed that the pyruvyl group is the critical binding determinant. Importantly, a single pyruvylated ManNAc monosaccharide can provide strong binding to the S-layer proteins, making it a promising structure for S-layer protein manipulation. The presence of

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acetyl esters and free amine did not have substantial effects on the binding of the various SLH proteins. Therefore, it is likely that the acetyl esters influence the composition of the S-layer in indirect manners by, for example, modulating the localization of other proteins involved in S-layer formation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b08857.

Preparation of the starting materials, procedures for the assembly of the oligosaccharides, and copies of NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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