Chemical Science

EDGE ARTICLE

Cite this: DOI: 10.1039/c3sc53362j

Synthesis of conjugation-ready zwitterionic oligosaccharides by chemoselective thioglycoside activation†

B. Schumann,[ab] R. Pragani,‡[a] C. Anish,[a] C. L. Pereira*[a] and P. H. Seeberger*[ab]

Received 7th December 2013
Accepted 23rd December 2013

DOI: 10.1039/c3sc53362j

www.rsc.org/chemicalscience

Commensal bacteria are ubiquitous inhabitants of mucosal surfaces and play an important role in promoting the maturation of the mammalian immune system. Zwitterionic polysaccharides (ZPSs) are found on the surface of certain commensal bacteria and exhibit important immunomodulatory activity. ZPSs are the first known carbohydrate antigens to induce an immune response by a T cell-dependent pathway. To understand the mechanism of their immunomodulatory activity, structurally-defined ZPS probes are needed. Here, we report the first total syntheses of repeating units of the two most prominent ZPSs, *S. pneumoniae* Sp1 (**1**) and *B. fragilis* PS A1 (**2**), and their immunological characterization after conjugation to reporter moieties. The introduction of a thioether-containing linker at an early stage of the synthesis called for establishing a method to chemoselectively activate thioglycosides in the presence of benzylthioethers. After oligosaccharide assembly, the same mild activation conditions were used in a novel way to introduce a benzyloxymethyl ether to cap the base-labile AAT residue, which allowed for completion of the syntheses. The appended thiol linkers enabled the conjugation of oligosaccharides **1** and **2** to glycan array and carrier protein moieties. Glycan array analysis revealed recognition of synthetic Sp1, but not PS A1, by antiserum against the native polysaccharide, demonstrating the applicability of conjugation-ready ZPS probes in biochemical settings. Further studies will give insight into the immunomodulatory properties of ZPSs.

Introduction

Bacterial capsular polysaccharides (CPSs) are important for the survival of bacteria in a mammalian host.[1] Since CPSs are found on the outermost layer of bacterial cells, these glycans interact with components of the immune system.[2] While CPSs of pathogenic bacteria are used in current vaccine formulations,[3] polysaccharides found on commensal or symbiotic bacteria contribute to the maturation of the host's immune system.[4]

Most bacterial CPSs are either uncharged or carry negative charges arising for example from uronic acid, phosphodiester or pyruvate moieties.[5] A small number of bacterial polysaccharides harbor repeating units with zwitterionic charge motifs. These zwitterionic polysaccharides (ZPSs) exhibit unique immunomodulatory activity and are commonly associated with commensalism.[4b,d,6] ZPSs are the first carbohydrate-only antigens to induce a T cell-dependent immune response through a major histocompatibility complex (MHC) class II dependent pathway.[6b,7] Furthermore, ZPSs affect the immune system through the stimulation of cytokine release by binding to toll-like receptor 2.[8] Interestingly, introducing zwitterionic charge motifs into non-zwitterionic polysaccharide-based vaccines has been shown to result in T cell activation and increased vaccine efficacy.[9]

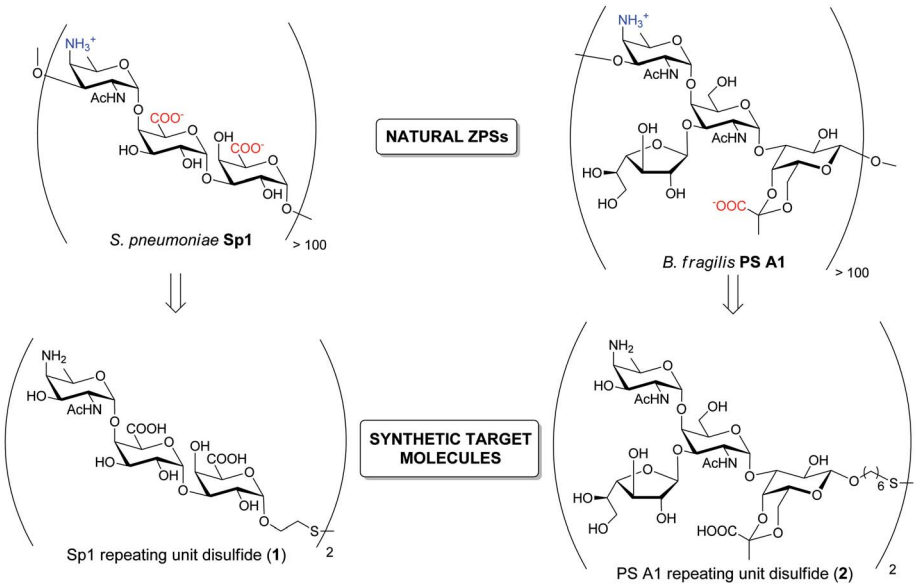
The most prominent and best-studied ZPS representatives are found on the surface of *Streptococcus pneumoniae* serotype 1 and *Bacteroides fragilis* (Scheme 1).[6d] Both bacteria colonize mucosal surfaces of healthy individuals. *B. fragilis* is a gut commensal and induces sterile abscesses upon intra-abdominal lesions during surgery.[4b] *S. pneumoniae* is found in the respiratory tract of healthy humans and can cause invasive disease upon colonization of otherwise sterile sites, especially in immunocompromised individuals.[1d] CPSs of both bacterial species are of high molecular weight[4f,7e] and harbor a variety of highly unusual monosaccharides, even for bacterial glycans.[5c] The repeating unit of *S. pneumoniae* Sp1 is a trisaccharide consisting of two D-galacturonic acid moieties and the rare aminosugar 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-AAT).[5a,10] The native *B. fragilis* PS A1 repeating unit is a branched tetrasaccharide, and the positive and negative

[a]Max Planck Institute of Colloids and Interfaces, 14424 Potsdam, Germany. E-mail: claneylebev.pereira@mpikg.mpg.de

[b]Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany. E-mail: peter.seeberger@mpikg.mpg.de

† Electronic supplementary information (ESI) available: Experimental procedures, a procedure for an NIS/TfOH-promoted control glycosylation using thioglycoside **3** and an oxoether-containing alcohol, supporting Fig. SI-1 and SI-2, and spectral characterization of all new compounds. See DOI: 10.1039/c3sc53362j

‡ National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, USA.



Scheme 1 Natural ZPSs and synthetic target molecules.

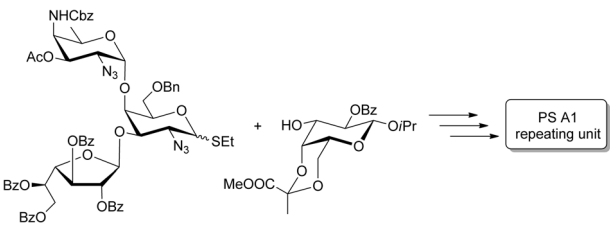
charges are provided by D-AAT and 4,6-*O*-pyruvalated D-galactose moieties, respectively.[11] In solution, bacterial ZPSs adopt an extended right-handed helical conformation with the positive and negative charges exposed to the environment.[12] The biological activity of these ZPSs has been shown to be dependent on their helical secondary structure.[12b,c] Studies using isolated ZPSs have greatly contributed to the understanding of this class of carbohydrates. However, the process of purification, fragmentation and labeling inevitably changes the structure of the glycan and may influence the immunological effects observed with ZPS probes. Furthermore, these studies are conducted on a heterogeneous mixture of ZPS fragments and thus, the minimal glycan size needed for inducing a T cell dependent immune response is not precisely known.[12c] Thus, defined synthetic zwitterionic oligosaccharides are necessary tools for studying the role of ZPSs in immunomodulation.

To date, several homogeneous ZPS fragments have been prepared by chemical synthesis, including a PS A1 tetrasaccharide and an Sp1 hexasaccharide.[13] While these glycans are valuable tools to study the structural requirements of ZPS recognition, they do not bear a linker capable of chemoselective conjugation to reporter moieties to investigate the mechanistic details of ZPS immunomodulation. In turn, forging an orthogonal linker at the reducing end enables the conjugation to carrier proteins, fluorophores, biotin, microarray surfaces and surface plasmon resonance (SPR) chips.[14] In most cases, synthetic glycans are equipped with amine-containing linkers at the reducing end to form adducts with suitable electrophiles. Thiol linkers have been used in the conjugation of oligosaccharides to proteins, gold nanoparticles and surfaces.[14h,15] However, the thiol moiety is usually introduced at the very end of a synthetic route due to incompatibilities with oxidation reactions in oligosaccharide assembly, such as thioglycoside activation.[14g,15,16] Thus, thiol-linked glycans have seen limited use for oligosaccharide conjugation chemistry due to their synthetic liability.

Results and discussion

Development of a chemoselective thioglycoside activation strategy

We targeted an *S. pneumoniae* Sp1 disulfide (**1**) and a *B. fragilis* PS A1 disulfide (**2**), in line with our efforts to synthesize homogeneous conjugation-ready ZPS fragments as tools to study ZPS biology (Scheme 1). The use of an amine-functionalized linker toward this end was precluded by the presence of free primary amines in both ZPSs, which would complicate site-selective conjugation. Thiol groups can be chemoselectively coupled with suitable electrophiles in the presence of free amines.[17] Thus, we targeted oligosaccharides that were equipped with a thiol linker at the reducing end of the fragments as shown in **1** and **2** from the outset.[18] Introduction of the thiol linker at an early stage of the synthesis was proposed as this approach could be translated to solid-phase synthesis that originates from the reducing end, and also renders the synthesis more convergent. However, in our earlier synthesis of the PS A1 tetrasaccharide repeating unit,[13a] the key [3 + 1]



Scheme 2 Key step in a previous PS A1 synthesis.[13a]

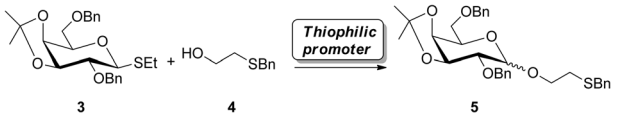
This journal is © The Royal Society of Chemistry 2014

glycosylation could only be executed using thioglycoside chemistry (Scheme 2). Literature precedents did not provide any indication as to whether a protected thiol would survive thioglycoside activation conditions.[14g,16]

Thus, known thioglycoside **3**[19] and thioether-containing alcohol **4**[20]were used in a model glycosylation to evaluate the chemoselectivity of different thioglycoside activation methods (Table 1).[21] The use of strong promoters, such as the well-known Ph_2SO/Tf_2O[22] combination or the more recently reported Me_2S_2/Tf_2O system,[23] resulted in product formation in 43% and 51% yield, respectively (Table 1, entries 1 and 2). While in both cases a considerable amount of the hydrolyzed thioglycoside could be identified as a major side product, thioether **4** could not be recovered when Ph_2SO/Tf_2O was used as an activator system (entry 1). In the presence of NIS/TfOH[24] as a promoter mixture, the glycosylation reaction did not proceed to completion (Table 1, entry 3), resulting in 54% yield of **5** and recovery of unreacted thioglycoside **3** and alcohol **4**. Employing 3 Å acid-washed molecular sieves instead of unwashed 3 Å molecular sieves did not improve the outcome of the glycosylation (Table 1, entry 4). Incomplete turnover in these reactions was unexpected because of the highly reactive nature of thioglycoside **3**.[25] Indeed, a test glycosylation between **3** and monobenzyl ethylene glycol instead of thioether **4** with NIS/TfOH led to complete conversion (see ESI†). Thus, it is proposed that in these glycosylation reactions (Table 1, entries 3 and 4) the electrophilic iodonium species is in part sequestered by the alkyl benzylthioether moiety in **4**, resulting in incomplete turnover.[14g] When MeOTf was used as a promoter in presence of an acid scavenger,[26] only traces of product were obtained (Table 1, entry 5). Methylation of the benzylthioether in **4** and **5** was observed instead, indicating that MeOTf does not discriminate between the thioglycoside and alkyl benzylthioether functional groups. Using mild activating agent DMTST[27] as a promoter provided glycoside **5** (Table 1, entry 6) in 76% yield, with hydrolysis of the glycosylating agent being the only observable side reaction. Thus, DMTST was found to be the best promoter for the chemoselective activation of thioglycoside **3** in the presence of the benzylthioether functionality found in **4**.

We next evaluated the substrate scope of the DMTST-mediated thioglycoside activation in the presence of benzylthioether **11** (Table 2).[28] Nucleophile **11** was used in glycosylation reactions with reactive thioglycosides **3** and **6**[29] using DMTST activation at low temperature, to provide glycosides **12** and **13** in 70% and 75% yield, respectively (Table 2, entries 1 and 2). Glycosylating agent **7**[30] (Table 2, entry 3) required reaction optimization due to the presence of the participating benzoyl ester protecting group at C2. Employing an excess of TTBPy (neutral conditions) led to the formation of high amounts of the respective orthoester as a side product, whereas benzylidene cleavage was observed when the scavenger was omitted (acidic conditions). It was found that using 1.0 to 1.2 equivalents of scavenger and two equivalents of DMTST produced a weakly

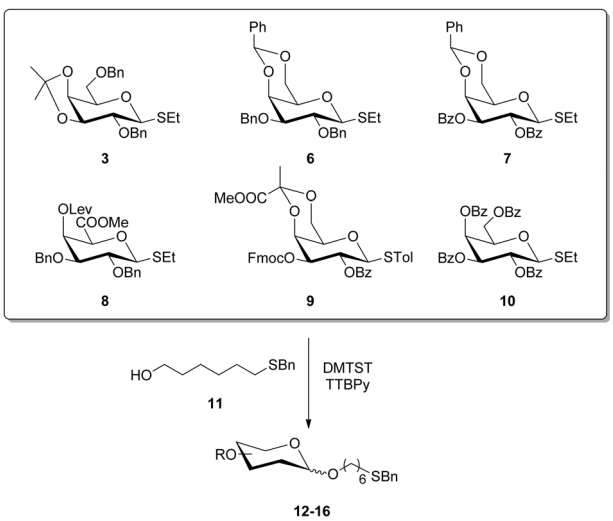
Table 1 Compatibility of thioglycoside activation methods with alkyl benzylthioether **4**



Entry[a]	Promoter (equiv.)[b,c]	Temp.	Yield,[21] %
1	Ph_2SO/Tf_2O (1.1/1.1), TTBPy (1.5)[d,e]	−60 °C to −10 °C	43[g]
2	Me_2S_2/Tf_2O (1.5/1.5), TTBPy (1.5)	−40 °C	51
3	NIS/TfOH (1.5/0.2)	−40 °C to 5 °C	54[h]
4	NIS/TfOH (1.5/0.2)[f]	−40 °C to 5 °C	42[h]
5	MeOTf (1.2), TTBPy (2.0)	0 °C to r.t.	<10[g]
6	DMTST (1.5), TTBPy (2.0)	0 °C	76

[a] 1.0 equiv. glycosylating agent, 1.5 equiv. alcohol **4**. [b] Reaction performed in CH_2Cl_2/Et_2O 1 : 3 (*v*/*v*). [c] 3 Å mol. sieves were used. [d] Reaction performed in CH_2Cl_2. [e] Pre-activation of glycosylating agent. [f] 3 Å-AW mol. sieves were used. [g] Thioether decomposed. [h] Reaction incomplete. DMTST = dimethyl(methylthio)sulfonium trifluoromethanesulfonate. MeOTf = methyl trifluoromethanesulfonate. NIS = *N*-iodosuccinimide. Tf_2O = trifluoromethanesulfonic anhydride. TfOH = trifluoromethanesulfonic acid. TTBPy = 2,4,6-tri-*tert*-butylpyridine.

Table 2 Scope and limitations of the chemoselective thioglycoside activation with DMTST/TTBPy



Entry[a]	Thioglycoside[c]	DMTST/ TTBPy, equiv.	Time/ temp.	Product (α : β)	Yield,[e] %
1	**3**	1.5/2.0	1 h/0 °C	**12** (1 : 1.6)	70
2	**6**	1.5/2.0	2 h/−10 °C	**13** (1 : 1.1)	75
3	**7**	2.0/1.2	1.5 h/r.t.	**14** (0 : 1)	70
4[b]	**8**	1.5/2.0	8 h/r.t.	**15** (4 : 3)	52[f]
5	**9**	1.8/1.1	16 h/r.t.	**16** (0 : 1)	58[f]
6	**10**	2.0/1.1	>100 h/r.t.	—	<20
7	**3**[d]	1.5/2.0	2 h/0 °C	**12** (1 : 1.6)	91

[a] Reaction performed in CH_2Cl_2. [b] Reaction performed in CH_2Cl_2/Et_2O 1 : 3 (v/v). [c] 1.0 equiv. glycosylating agent, 1.5 equiv. alcohol **11**. [d] 1.4 equiv. glycosylating agent, 1.0 equiv. alcohol **11**. [e] Isolated yields. [f] Isolated yield after consecutive step.

acidic environment that yielded glycoside **14** without any major side reactions. Activation of galacturonic acid thioglycoside **8** (see ESI†) in the presence of alcohol **11** provided glycoside mixture **15** in 52% yield over two steps after removal of the C4 Lev ester (Table 2, entry 4). The moderate yield is consistent with previous reports on the low reactivity of galacturonic acid glycosylating agents.[13b,31] Nevertheless, complete chemoselectivity was observed in the activation of glycosylating agent **8**, leaving the alkyl benzylthioether moiety intact.

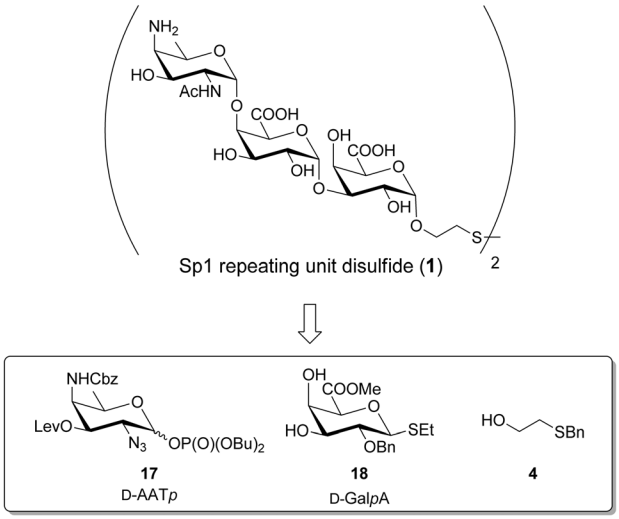
As a brief look at other anomeric thioether leaving groups, the chemoselective activation of *p*-toluyl thioglycoside **9**[13a] in the presence of the benzylthioether found in **11** was executed (Table 2, entry 5). It is known that aryl thioglycosides are less readily activated by DMTST than alkyl thioglycosides.[32] However, even toluyl thioglycoside **9** was chemoselectively activated by DMTST, giving pyruvylated galactoside **16** in 58% yield after removal of the C3 Fmoc group. Finally, perbenzoylated galactose thioglycoside **10**[33] was employed as a highly electron-deficient glycosylating agent (Table 2, entry 6). Even after prolonged stirring at room temperature, only very little consumption of the starting material was observed. Increased amounts of DMTST or higher reaction temperatures did not result in the formation of the desired product, but led to decomposition of the thioether group in **11** (data not shown). We speculated that the reactivities of both sulfur atoms in thioglycoside **10** and thioether **11** are comparable,[39] and thus, both compete for DMTST, leading to the decomposition of **11**.

To fully confirm the chemoselectivity of DMTST-mediated thioglycoside activation, alcohol **11** was reacted with an excess of thioglycoside **3** and DMTST (Table 2, entry 7). Nearly full conversion of **11** was achieved, giving glycoside **12** in 91% yield without affecting the alkyl benzylthioether. Taken together, these results suggest that a wide range of thioglycosides of different reactivities can be efficiently activated by DMTST in the presence of a benzylthioether. However, highly deactivated thioglycosides, such as **10**, do not couple under these conditions.

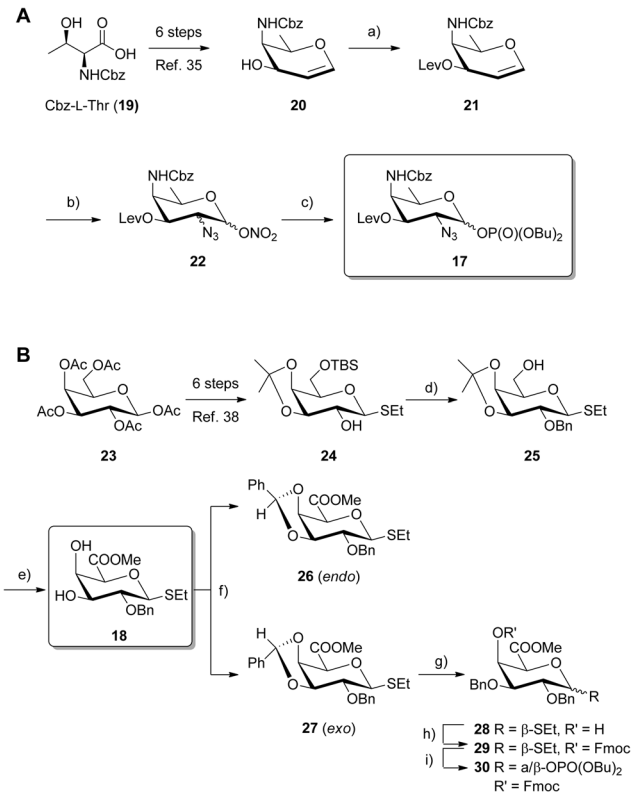
Total synthesis of a conjugation-ready Sp1 repeating unit trisaccharide

With a method to conveniently introduce a benzylthioether at an early stage in hand, attention was directed toward the synthesis of conjugation-ready zwitterionic oligosaccharides. We envisaged that trisaccharide dimer **1**, derived from *S. pneumoniae* serotype 1 CPS, could be assembled from AAT building block **17**, galacturonic acid diol **18**, and thioether **4** (Scheme 3).

AAT building block **17** was prepared *via* a *de novo* synthetic route recently established in our lab, using Cbz-L-threonine **19** as a chiral, inexpensive precursor (Scheme 4, panel A).[35] Alcohol **20** was condensed with levulinic acid to give ester **21** in 92% yield. Azidonitration provided glycosyl nitrate **22** as an inseparable 4 : 1 *galacto*/*talo* isomeric mixture.[35] Nucleophilic displacement of the anomeric nitrate with cesium dibutyl phosphate provided AAT phosphate **17** in 37% yield over two steps.[36] Compared to already known AAT imidates,[13b,35,37] we anticipated that an AAT phosphate glycosylating agent would



Scheme 3 Synthesis of building blocks for the assembly of Sp1 repeating unit disulfide **1**.



Scheme 4 Synthesis of building blocks for the assembly of Sp1 repeating unit disulfide **1**. Reagents and conditions: (a) LevOH, EDC, DMAP, pyr., CH_2Cl_2, r.t., 92%; (b) CAN, NaN_3, CH_3CN, −20 °C; (c) $CsOPO(OBu)_2$, DMF, r.t., 37% (two steps from **21**); (d) i. BnBr, NaH, DMF, 0 °C to r.t.; ii. TBAF, THF, 0 °C to r.t., 81% (two steps); (e) i. $PhI(OAc)_2$, TEMPO, CH_2Cl_2, H_2O, 0 °C to r.t., 3 h; ii.AcCl, MeOH, 0 °C to r.t., 60% (two steps); (f) $PhCH(OMe)_2$, TsOH, CH_3CN, r.t., 92% (1 : 1 *endo*/*exo*); (g) TES, TFA, TFAA, 0 °C to r.t., 65%; (h) FmocCl, pyr., 0 °C to r.t., 90%; (i) NIS, $HOPO(OBu)_2$, CH_2Cl_2, r.t., 89%. CAN = ceric ammonium nitrate. DMAP = 4-(dimethylamino)pyridine. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. TEMPO = 2,2,6,6-tetramethylpiperidine 1-oxyl. TES = triethylsilane. TFA = trifluoroacetic acid. TFAA = trifluoroacetic anhydride. TsOH = *p*-toluylsulfonic acid.

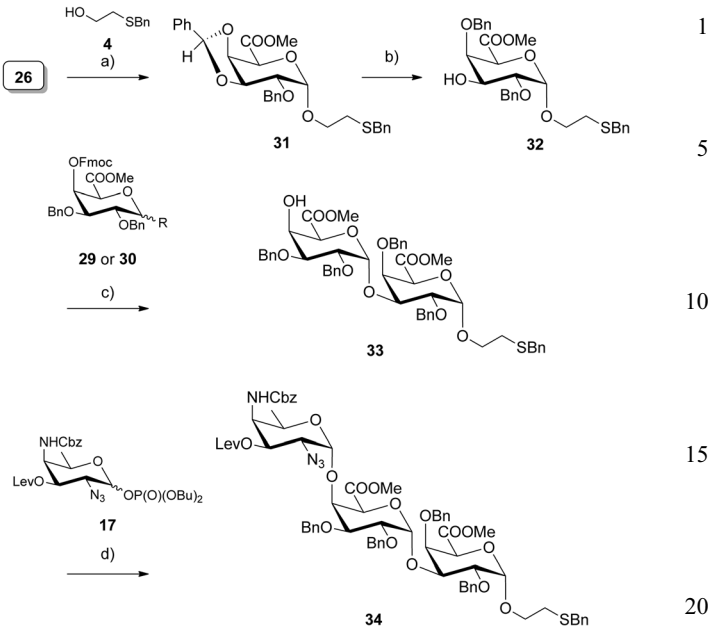
This journal is © The Royal Society of Chemistry 2014

display enhanced stability towards decomposition while reducing the number of synthetic steps for preparation.

The native repeating unit of *S. pneumoniae* serotype 1 CPS contains two galacturonic acid residues that are glycosylated at either the C3 or C4 positions, respectively.[10] For the generation of differentially C3–OH/C4–OH-functionalized GalA building blocks, diol **18** was targeted as a common intermediate to minimize the total number of synthetic steps. Commercially available galactose pentaacetate **23** served as the starting point for the synthesis of diol **18**. Alcohol **24** was synthesized according to a literature procedure from **23** (Scheme 4, panel B).[38] Benzyl protection of the C2-hydroxyl group followed by TBS deprotection at the C6 position afforded alcohol **25** in 81% yield over two steps. TEMPO-mediated oxidation and subsequent treatment with anhydrous hydrogen chloride in methanol then gave galacturonic acid diol **18** in 60% yield over two steps.

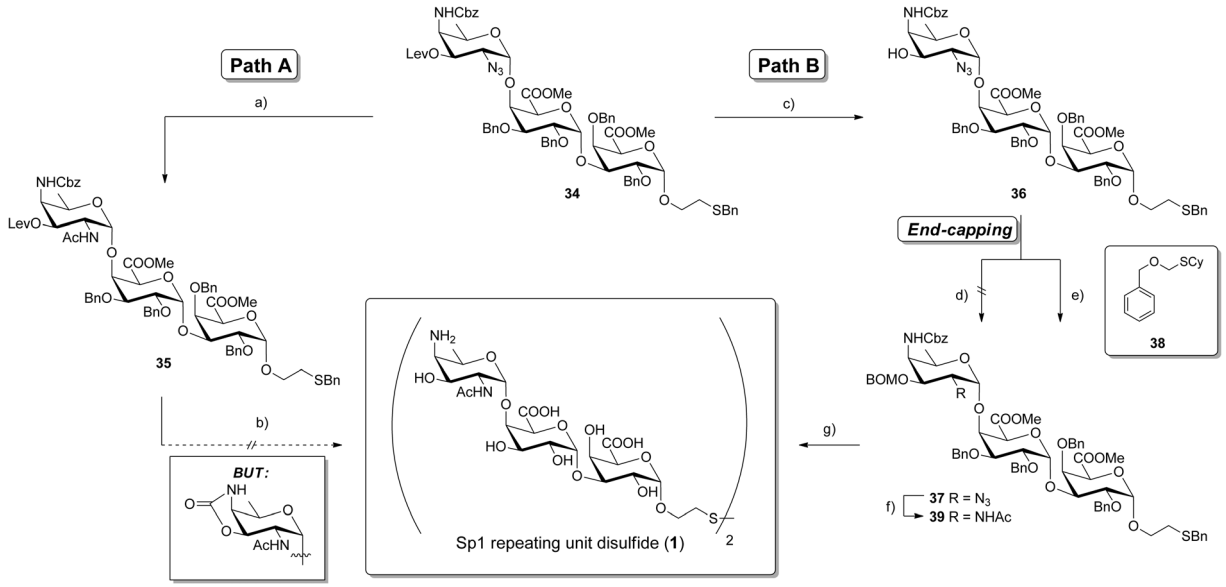
At this stage, attempts to introduce benzyl protecting groups under either basic (BnBr, NaH) or strongly acidic conditions (benzyl trichloroacetimidate, TfOH) resulted exclusively in the decomposition of compound **18**. Therefore, a two-step procedure was envisioned for the selective benzyl protection of either the C3- or C4-hydroxyl groups *via* intermediate 3,4-*O*-benzylidene acetals. In the first step, diol **18** was treated with benzaldehyde dimethyl acetal under weakly acidic conditions to furnish isomeric benzylidene acetals **26** and **27** in a 1 : 1 ratio in 92% overall yield. Since the regioselectivity of the second step, a reductive benzylidene acetal ring-opening, is dependent on the benzylidene configuration,[39] the generation of an equimolar mixture of both epimers was important for the synthesis of both galacturonic acids in Sp1 target trisaccharide **1**.[40] The configurations of *endo*-acetal **26** and *exo*-acetal **27** were confirmed by HH-NOESY NMR spectroscopy (see ESI†).[39f,41] As anticipated, *exo*-acetal **27** was ring-opened using TES and TFA to give dibenzyl ether **28** in 65% yield and complete regioselectivity. Fmoc protection of the free C4 hydroxyl group furnished carbonate **29** in 90% yield. To enable the screening of different glycosylating agents at a later stage, thioglycoside **29** was transformed into glycosyl phosphate **30** in 89% yield.

With the building blocks in hand, the assembly of Sp1 trisaccharide **1** was undertaken (Scheme 5). DMTST-mediated glycosylation of thioglycoside **26** with thioether-containing alcohol **4** in THF gave glycoside **31** in 73% yield with modest α-selectivity. The low diastereoselectivity of this glycosylation is consistent with the results usually obtained with highly nucleophilic, primary alcohols.[42] A completely regioselective ring-opening of the *endo*-benzylidene in **31** using $BH_3\cdot NMe_3$ and $AlCl_3$ gave alcohol **32** in 70% yield.[39c,43] Glycosylation of alcohol **32** with glycosyl phosphate **30** (80% yield) with subsequent Fmoc removal (68% yield) provided digalacturonic acid **33** in a 3 : 1 α/β anomeric ratio. The use of ethereal solvents in the glycosylation reaction did not alter the diastereoselectivity. DMTST-mediated glycosylation of thioglycoside **29** with alcohol **32** was unable to undergo complete conversion, presumably due to the low reactivity of both alcohol and glycosylating agent.[31b] Glycosylation of alcohol **33** with AAT phosphate **17** proceeded uneventfully using TMSOTf as the promoter. Thus, trisaccharide **34** was obtained in 85% yield and with complete α-selectivity, highlighting the suitability of AAT phosphate building block **17** in oligosaccharide synthesis.



Scheme 5 Assembly of Sp1 trisaccharide **34**. Reagents and conditions: (a) 1.5 equiv. **4**, DMTST, TTBPy, THF, 0 °C to r.t., 73% (1.7 : 1 α/β); (b) $BH_3\cdot NMe_3$, $AlCl_3$, THF, r.t., 70%. (c) i. 1.5 equiv. **30**, TBSOTf, CH_2Cl_2, 80% (3 : 1 α/β) or 2.3 equiv. **29**, DMTST, TTBPy, Et_2O/CH_2Cl_2 3:1, r.t., 50% (3 : 1 α/β) ii. Et_3N, CH_2Cl_2, r.t., 68%; (d) 1.5 equiv. **17**, TMSOTf, CH_2Cl_2, 0 °C, 85% (>19 : 1 α/β). TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate. TMSOTf = trimethylsilyl trifluoromethanesulfonate.

With the fully protected Sp1 trisaccharide core (**34**) in hand, global deprotection strategies were assessed. Reductive acetylation of the azide followed by sequential removal of all protecting groups present in **34** was attempted (Scheme 6, path A). Thus, acetamide **35** was obtained in 72% yield after treatment of **34** with thioacetic acid and pyridine.[13a,44] It was now important to hydrolyze the methyl esters prior to Birch reduction to prevent β-elimination of the galacturonic acid moieties under the harshly basic Birch conditions.[45] However, saponification of the methyl and Lev esters using either NaOH or Cs_2CO_3 in THF, water and methanol resulted in the concomitant cyclization of the AAT-NHCbz group to the corresponding cyclic carbamate. This side reaction has been associated with Cbz-protected AAT moieties previously.[13a,b] We envisaged that a capping step of the AAT C3–OH would allow us to circumvent cyclization of the Cbz moiety (Scheme 6, path B). Capping of the AAT C3 hydroxyl group should ideally involve a stable, permanent protecting group. Thus, Lev deprotection of trisaccharide **34** gave C3 alcohol **36** in quantitative yield. Introduction of benzylic ethers under highly basic conditions was excluded due to the base-lability of alcohol **36**.[46] Benzyloxymethyl (BOM) ethers can be introduced under mild, weakly basic conditions.[47] Unfortunately, treatment of trisaccharide **36** with BOMCl and DIPEA in refluxing CH_2Cl_2 led to decomposition of the starting material. To enable the introduction of the BOM acetal moiety under milder conditions, we adapted a method described for the generation of formyl acetals by activation of *S*,*O*-acetal



Scheme 6 Global deprotection to yield Sp1 repeating unit disulfide **1**. Reagents and conditions: (a) AcSH, pyr., r.t., 72%.; (b) NaOH, H_2O, THF, 0 °C to r.t.; or Cs_2CO_3, H_2O, THF, 0 °C to r.t.; (c) $H_4N_2 \cdot H_2O$, HOAc, pyridine, CH_2Cl_2, r.t., quant.; (d) BOMCl, iPr_2NEt, CH_2Cl_2, reflux; (e) **38**, DMTST, TTBPy, CH_2Cl_2, 0 °C–15 °C, 84%; (f) AcSH, pyridine, 0 °C to r.t., 72%; (g) i. NaOH, H_2O, THF, MeOH, 0 °C to r.t.; ii. Na, NH_3, *t*BuOH, THF, −78 °C; then air, r.t., 93% (two steps).

precursors.[48] Therefore, we used our own established conditions for the chemoselective activation of thioglycosides in the presence of thioethers to develop a procedure for introducing a BOM group under mild conditions. *S*,*O*-acetal **38**, readily prepared from commercially available reagents (see ESI†), was chosen as a suitable BOM precursor with a reactive *S*-cyclohexyl leaving group.[32] DMTST-mediated activation of *S*,*O*-acetal **38** under buffered reaction conditions and at low temperature converted alcohol **36** into the desired BOM-protected trisaccharide **37** in 83% yield. As anticipated, the alkyl benzylthioether remained unharmed despite the use of excess BOM precursor **38** as well as DMTST. To our knowledge, this presents the mildest method so far to introduce a benzyloxymethyl ether into a complex substrate like **36**.

With capped trisaccharide **37** in hand, the stage was set for the completion of the synthesis. Reductive acetylation provided acetamide **39** in 72% yield. With the AAT C3 hydroxyl group capped, saponification of both methyl esters with NaOH in THF, methanol and water proceeded without any side reactions. Finally, Birch reduction was carried out to remove all benzyl ethers as well as the Cbz and BOM groups to afford disulfide **1** in 93% yield over two steps after size exclusion chromatography.[49] The identity of disulfide **1** was confirmed unambiguously by comparison of our analytical data with published results.[13b,c] Particularly, the presence of three α-anomeric linkages can be deduced from the small $^3J_{H,H}$ coupling constants of the anomeric protons (3.8 Hz each; see ESI†) in the ^{1}H NMR spectrum.

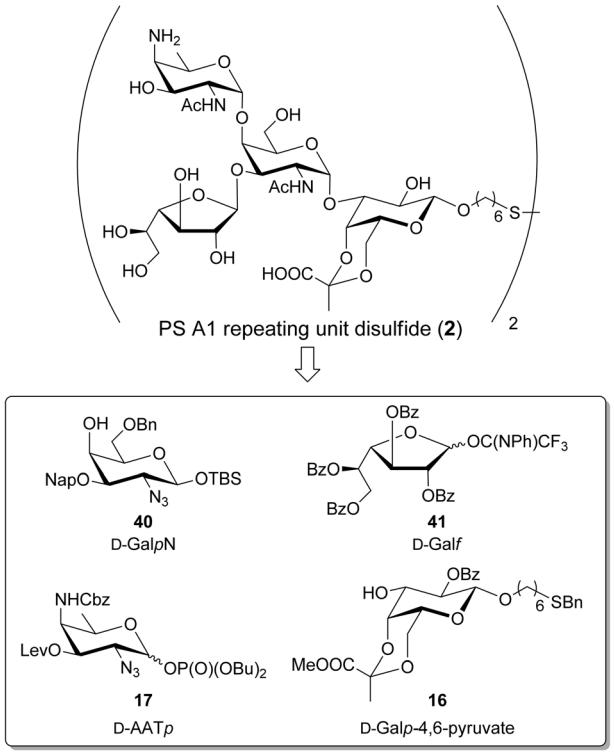
Total synthesis of a conjugation-ready PS A1 repeating unit tetrasaccharide

Based on the lessons learned synthesizing the Sp1 repeating unit disulfide **1**, we took on the preparation of PS A1 repeating unit disulfide **2**. To assemble the tetrasaccharide backbone of **2**, we adapted the previously established [3 + 1] strategy and based our retrosynthetic analysis on building blocks **40**, **41**, **17** and **16** (Scheme 7).[13a,35]

Synthesis of PS A1 repeating unit disulfide **2** commenced with the glycosylation of AAT glycosylating agent **17** and galactosamine nucleophile **40** to give disaccharide **42** in 77% yield and 19 : 1 α/β selectivity (Scheme 8). Removal of the Nap group (84% yield) was followed by glycosylation of disaccharide **43** with galactofuranose imidate **41** to provide trisaccharide **44** in 90% yield. Modification of the trisaccharide reducing end included TBS deprotection to give lactol **45** (89% yield), formation of the corresponding trifluoroacetimidate and thioglycoside installation to obtain glycosylating agent **46** in 75% yield over two steps. Chemoselective glycosylation of thioglycoside **46** with thioether-containing alcohol **16** (see Table 2) was performed using DMTST to give tetrasaccharide **47** in 57% yield as the sole diastereomer without affecting the alkyl benzylthioether. Lev deprotection provided alcohol **48** in 96% yield, which was capped using novel reagent **38** and DMTST to give BOM protected tetrasaccharide **49** in 87% yield. Conversion of both azides to the corresponding acetamides with AcSH/pyridine produced diamide **50** in 60% yield.

Global deprotection of tetrasaccharide **50** was achieved in a straightforward two-step procedure. Saponification of all esters followed by Birch reduction provided fully-deprotected disulfide **2** in 88% yield over two steps after size exclusion chromatography. No major side reactions were observed during global deprotection, highlighting the feasibility of introducing a thioether at an early synthetic stage. The identity of disulfide **2** was confirmed by comparing the analytical data of **2** with published data on a similar synthetic PS A1 repeating unit.[13a]

This journal is © The Royal Society of Chemistry 2014

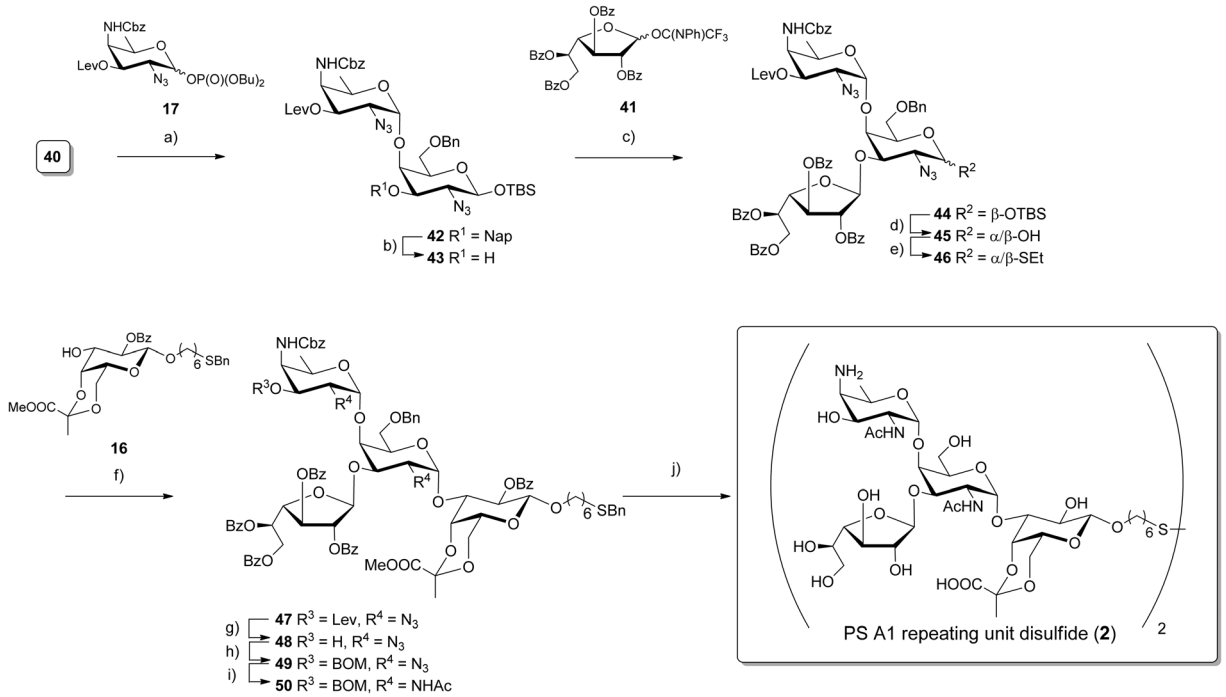


Scheme 7 Retrosynthesis of PS A1 repeating unit disulfide **2**.

Recognition of synthetic oligosaccharides by anti-ZPS antibodies

With both conjugation-ready oligosaccharides **1** and **2** in hand, we set out to evaluate the recognition of these ZPS fragments by components of the immune system by glycan microarray.[50] Oligosaccharides were reduced *in situ*[51] and printed onto maleimide-functionalized glass slides.[52] To assess the role of selected monosaccharides on glycan recognition, galacturonic acid and 4,6-*O*-pyruvalated galactose, prepared by global deprotection of monosaccharides **15** and **16** (see ESI†), were included in the experiment (Fig. 1A). Binding was assessed using rabbit antisera against either Sp1 polysaccharide or whole *Bacteroides fragiles* bacteria (Fig. 1B and C). A robust interaction was found between synthetic Sp1 and antiserum against the native polysaccharide, indicating that the synthetic repeating unit efficiently presents epitopes found in the native structure. Binding was observable at high dilutions of up to 1 : 6000, underlining the potency of synthetic Sp1 to structurally mimic the polysaccharide (see ESI, Fig. SI-1A†). Conversely, galacturonic acid alone was not recognized by Sp1 antiserum, suggesting that GalA alone is not an immunodominant epitope.

Negligible interaction was found between anti-*B. fragilis* antiserum and synthetic PS A1 repeating unit **2** (Fig. 1B and C). Binding of pyruvylated galactose was equally weak. In contrast, native PS A1 polysaccharide was recognized by the antiserum (see ESI, Fig. SI-1B†). We speculate that native epitopes recognized by the antiserum are not present in the synthetic repeating unit. Recently, both genomic and mass spectrometric analyses indicated that



Scheme 8 Synthesis of PS A1 repeating unit disulfide **2**. Reagents and conditions: (a) 1.5 equiv. **17**, TMSOTf, CH_2Cl_2, 0 °C, 77% (19:1 α/β); (b) DDQ, MeOH, CH_2Cl_2, 0 °C to r.t., 84%; (c) 1.4 equiv. **41**, TMSOTf, CH_2Cl_2, −30 °C, 90% (>19:1 β/α); (d) TBAF, AcOH, THF, 0 °C to r.t., 89%; (e) i. $F_3CC(NPh)Cl$, Cs_2CO_3, CH_2Cl_2, r.t.; ii. EtSH, TfOH, CH_2Cl_2, 0 °C, 76% (two steps); (f) 2.0 equiv. **16**, DMTST, TTBPy, CH_2Cl_2, r.t., 57% (>19:1 α/β); (g) H_4N_2, pyridine, AcOH, CH_2Cl_2, r.t., 96%; (h) **38**, DMTST, TTBPy, CH_2Cl_2, 0 °C–10 °C, 87%; (i) AcSH, pyr., r.t., 60%; (j) i. NaOH, H_2O, THF, MeOH, 0 °C to r.t.; ii. Na, NH_3, *t*BuOH, THF, −78 °C; then air, rt, 88% (two steps).

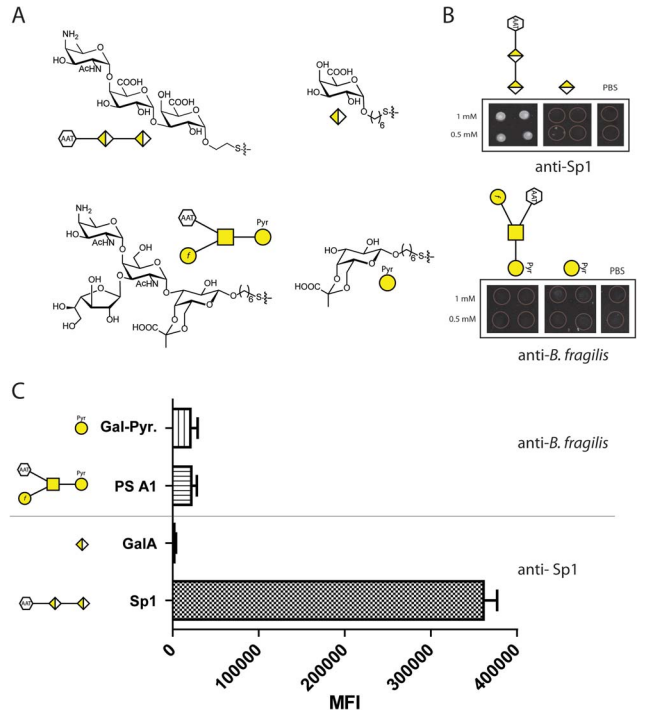


Fig. 1 Microarray analysis of synthetic zwitterionic oligosaccharides. Glycan disulfides were reduced and spotted onto maleimide-functionalized glass slides in different concentrations. Binding was assessed by incubation with rabbit antisera against Sp1 polysaccharide or entire *B. fragilis* bacteria and fluorescently labeled anti-rabbit secondary antibodies. (A) Graphical depiction of printed structures. (B) Representative microarray experiment of synthetic Sp1 (upper panel) and PS A1 (lower panel) structures. Spots without detectable signals are marked with a circle. (C) Quantification of the results obtained in B. Values represent mean ± standard deviation of **4** spots of the same concentration, normalized to buffer spots. MFI = mean fluorescence intensity. PBS = phosphate buffered saline.

D-AAT is found at the reducing end of the natural repeating unit of several polysaccharides, including native Sp1 and the non-serotype-specific *S. pneumoniae* lipoteichoic acid.[10c,53] Since CPS biosynthesis pathways are often conserved among species,[54] it may be speculated that D-AAT is also the first monosaccharide in the natural PS A1 repeating unit.[54d] Thus, tetrasaccharide **2** may not be recognized by *B. fragilis* antiserum because it spans the wrong frameshift of the natural polysaccharide. Curiously, Sp1 trisaccharide **1** displays D-AAT in a similar fashion and is strongly bound by the respective antiserum, suggesting that further structural properties govern ZPS recognition.

To further demonstrate that the generated thiol-linked oligosaccharides can be coupled to thiol-reactive moieties, we generated a protein conjugate of PS A1 tetrasaccharide **2** (see ESI, Fig. SI-2A†). After disulfide reduction, the free thiol form of **2** was incubated with bromoacetylated[15c] bovine serum albumin (BSA) to generate a conjugate that harbored on average 3.2 synthetic PS A1 molecules per BSA molecule (Fig. SI-2B and C†). Thus we conclude that the synthetic zwitterionic oligosaccharides presented here are indeed conjugation-ready and can be used for further immunological studies. Detailed biological evaluation of these oligosaccharides is currently underway.

Conclusions

Amine-containing linkers, commonly used to couple synthetic oligosaccharides to reporter molecules or carrier proteins, are not compatible with zwitterionic oligosaccharides Sp1 and PS A1. These molecules already contain free primary amines leading to a chemoselectivity problem during conjugation reactions. Our approach to generating conjugation-ready homogeneous Sp1 (**1**) and PS A1 (**2**) oligosaccharides relied on known orthogonal conjugation conditions that chemoselectively couple thiols and electrophiles in the presence of a free amine.[17] We demonstrated that benzylthioethers can be introduced early in the synthesis of oligosaccharides **1** and **2** without considerably decreasing the variability of chemical transformations. This approach depended on the discovery that thioglycosides can generally be activated by DMTST without affecting thioethers found elsewhere in the molecule. This strategy will influence the generation of other disulfide conjugation-ready oligosaccharides by solution and solid phase synthesis. The approach was illustrated by the total syntheses of Sp1 (**1**) and PS A1 (**2**) conjugation-ready oligosaccharides. To demonstrate the applicability of these conjugation-ready zwitterionic oligosaccharides in biochemical settings, saccharides **1** and **2** were immobilized onto functionalized glass slides after disulfide reduction. Glycan array revealed that synthetic Sp1 trisaccharide **1** is recognized by an antiserum against the native polysaccharide. This is the first demonstration of an interaction between a synthetic ZPS fragment and a component of the immune system. In contrast, neither PS A1 tetrasaccharide **2** nor any monosaccharide screened were found to interact with antisera against Sp1 or *B. fragilis*, which was not caused by insufficient immobilization. The structural details governing the binding behavior of synthetic ZPS fragments are under investigation and will be reported in due course. Conjugation-ready oligosaccharides **1** and **2** will be used to shed light onto the mechanisms of immunomodulation by ZPS.

Acknowledgements

The authors thank Prof. Dennis Kasper (Harvard Medical School) for anti-*B. fragilis* antiserum and PS A1 polysaccharide. We thank the Max Planck Society, the German Federal Ministry of Education and Research (BMBF) and the Körber Foundation for generous financial support. This work was supported by a Kekulé doctoral fellowship by the Fonds der Chemischen Industrie (B.S.) and a postdoctoral fellowship by the Alexander von Humboldt foundation (R.P.). We thank Dr Andreas Schäfer (Freie Universität Berlin) for NOESY-NMR measurements. We thank Dr Farhan Bou Hamdan and Dr Sharavathi Guddehalli Parameswarappa for helpful discussions, Christopher Martin and Eva Settels for MALDI measurements and Andreas Geissner and Melanie Leddermann for help with microarray experiments.

Notes and references

1 (*a*) M. J. Coyne, M. Chatzidaki-Livanis, L. C. Paoletti and L. E. Comstock, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**(35),

 This journal is © The Royal Society of Chemistry 2014

13099–13104; (*b*) S. Merino and J. M. Tomás, in *Encyclopedia of Life Sciences (ELS)*, John Wiley & Sons, Chichester, 2010; (*c*) C. M. Macleod and M. R. Krauss, *J. Exp. Med.*, 1950, **92**, 1; (*d*) A. D. Magee and J. Yother, *Infect. Immun.*, 2001, **69**, 3755; (*e*) S. L. Reckseidler-Zenteno, R. DeVinney and D. E. Woods, *Infect. Immun.*, 2005, **73**, 1106; (*f*) E. B. Troy, V. J. Carey, D. L. Kasper and L. E. Comstock, *J. Bacteriol.*, 2010, **192**, 5832; (*g*) C. H. Liu, S. M. Lee, J. M. VanLare, D. L. Kasper and S. K. Mazmanian, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3951; (*h*) C. L. Sears, *Anaerobe*, 2012, **18**, 192; (*i*) J. S. Nanra, S. M. Buitrago, S. Crawford, J. Ng, P. S. Fink, J. Hawkins, I. L. Scully, L. K. McNeil, J. M. Aste-Amezaga, D. Cooper, K. U. Jansen and A. S. Anderson, *Hum. Vaccines Immunother.*, 2012, **9**, 480.

2 (*a*) D. Corbett and I. S. Roberts, *F1000 Biol. Rep.*, 2009, **1**, 30; (*b*) F. Y. Avci and D. L. Kasper, *Annu. Rev. Immunol.*, 2010, **28**, 107; (*c*) Q. Vos, A. Lees, Z. Q. Wu, C. M. Snapper and J. J. Mond, *Immunol. Rev.*, 2000, **176**, 154.

3 (*a*) Y. S. Ovodov, *Biochemistry*, 2006, **71**, 955; (*b*) B. Schumann, C. K. Anish, C. L. Pereira and P. H. Seeberger, *Biotherapeutics: Recent Developments using Chemical and Molecular Biology, RSC Drug Discovery Series No. 36*, ed. L. Jones and A. J. McKnight, RSC Publishing, Cambridge, 2013, ch. 3, pp. 68–104.

4 (*a*) I. Jarchum and E. G. Pamer, *Curr. Opin. Immunol.*, 2011, **23**, 353; (*b*) N. K. Surana and D. L. Kasper, *Immunol. Rev.*, 2012, **245**, 13; (*c*) S. K. Mazmanian, C. H. Liu, A. O. Tzianabos and D. L. Kasper, *Cell*, 2005, **122**, 107; (*d*) E. B. Troy and D. L. Kasper, *Front. Biosci., Landmark Ed.*, 2010, **15**, 25; (*e*) Y. K. Lee and S. K. Mazmanian, *Science*, 2010, **330**, 1768; (*f*) W. M. Kalka-Moll, A. O. Tzianabos, Y. Wang, V. J. Carey, R. W. Finberg, A. B. Onderdonk and D. L. Kasper, *J. Immunol.*, 2000, **164**, 719.

5 (*a*) J. P. Kamerling, *Streptococcus pneumoniae*, ed. A. Tomasz, Mary Ann Liebert, Inc., Larchmont, 2000, pp. 81–114; (*b*) I. W. Sutherland, *Int. Dairy J.*, 2001, **11**, 663; (*c*) A. Adibekian, P. Stallforth, M.-L. Hecht, D. B. Werz, P. Gagneux and P. H. Seeberger, *Chem. Sci.*, 2011, **2**, 337.

6 (*a*) P. J. Sansonetti, *Mucosal Immunol.*, 2011, **4**, 8; (*b*) T. L. Stephen, L. Groneck and W. M. Kalka-Moll, *Int. J. Microbiol.*, 2010, **2010**, 917075; (*c*) S. K. Mazmanian, J. L. Round and D. L. Kasper, *Nature*, 2008, **453**, 620; (*d*) A. O. Tzianabos, A. B. Onderdonk, B. Rosner, R. L. Cisneros and D. L. Kasper, *Science*, 1993, **262**, 416.

7 (*a*) W. M. Kalka-Moll, A. O. Tzianabos, P. W. Bryant, M. Niemeyer, H. L. Ploegh and D. L. Kasper, *J. Immunol.*, 2002, **169**, 6149; (*b*) F. Stingele, B. Corthesy, N. Kusy, S. A. Porcelli, D. L. Kasper and A. O. Tzianabos, *J. Immunol.*, 2004, **172**, 1483; (*c*) B. A. Cobb, Q. Wang, A. O. Tzianabos and D. L. Kasper, *Cell*, 2004, **117**, 677; (*d*) C. D. Velez, C. J. Lewis, D. L. Kasper and B. A. Cobb, *Immunology*, 2009, **127**, 73; (*e*) T. L. Stephen, M. Fabri, L. Groneck, T. A. Rohn, H. Hafke, N. Robinson, J. Rietdorf, D. Schrama, J. C. Becker, G. Plum, M. Kronke, H. Kropshofer and W. M. Kalka-Moll, *PLoS Pathog.*, 2007, **3**.

8 (*a*) A. Wack and S. Gallorini, *Immunopharmacol. Immunotoxicol.*, 2008, **30**, 761; (*b*) Q. Wang, R. M. McLoughlin, B. A. Cobb, M. Charrel-Dennis, K. J. Zaleski, D. Golenbock, A. O. Tzianabos and D. L. Kasper, *J. Exp. Med.*, 2006, **203**, 2853.

9 (*a*) S. Gallorini, F. Berti, G. Mancuso, R. Cozzi, M. Tortoli, G. Volpini, J. L. Telford, C. Beninati, D. Maione and A. Wack, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 17481; (*b*) S. Gallorini, F. Berti, P. Parente, R. Baronio, S. Aprea, U. D'Oro, M. Pizza, J. L. Telford and A. Wack, *J. Immunol.*, 2007, **179**, 8208.

10 (*a*) B. Lindberg, B. Lindqvist, J. Lonngren and D. A. Powell, *Carbohydr. Res.*, 1980, **78**, 111; (*b*) C. J. M. Stroop, Q. W. Xu, M. Retzlaff, C. Abeygunawardana and C. A. Bush, *Carbohydr. Res.*, 2002, **337**, 335; (*c*) S. D. Bentley, D. M. Aanensen, A. Mavroidi, D. Saunders, E. Rabbinowitsch, M. Collins, K. Donohoe, D. Harris, L. Murphy, M. A. Quail, G. Samuel, I. C. Skovsted, M. S. Kaltoft, B. Barrell, P. R. Reeves, J. Parkhill and B. G. Spratt, *PLoS Genet.*, 2006, **2**, 262.

11 H. Baumann, A. O. Tzianabos, J. R. Brisson, D. L. Kasper and H. J. Jennings, *Biochemistry*, 1992, **31**, 4081.

12 (*a*) Y. Wang, W. M. Kalka-Moll, M. H. Roehrl and D. L. Kasper, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 13478; (*b*) L. S. C. Kreisman, J. H. Friedman, A. Neaga and B. A. Cobb, *Glycobiology*, 2007, **17**, 46; (*c*) A. Tzianabos, J. Y. Wang and D. L. Kasper, *Carbohydr. Res.*, 2003, **338**, 2531; (*d*) Y. H. Choi, M. H. Roehrl, D. L. Kasper and J. Y. Wang, *Biochemistry*, 2002, **41**, 15144.

13 (*a*) R. Pragani and P. H. Seeberger, *J. Am. Chem. Soc.*, 2011, **133**, 102; (*b*) A. E. Christina, L. J. van den Bos, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codee, *J. Org. Chem.*, 2011, **76**, 1692; (*c*) X. Y. Wu, L. N. Cui, T. Lipinski and D. R. Bundle, *Chem. – Eur. J.*, 2010, **16**, 3476; (*d*) I. Iynkkaran and D. R. Bundle, *Carbohydr. Res.*, 2013, **378**, 26.

14 (*a*) O. Calin, S. Eller, H. S. Hahm and P. H. Seeberger, *Chem. – Eur. J.*, 2013, **19**, 3995; (*b*) Y. E. Tsvetkov, M. Burg-Roderfeld, G. Loers, A. Arda, E. V. Sukhova, E. A. Khatuntseva, A. A. Grachev, A. O. Chizhov, H. C. Siebert, M. Schachner, J. Jimenez-Barbero and N. E. Nifantiev, *J. Am. Chem. Soc.*, 2012, **134**, 426; (*c*) C. E. Martin, M. W. Weishaupt and P. H. Seeberger, *Chem. Commun.*, 2011, **47**, 10260; (*d*) M. A. Oberli, M. L. Hecht, P. Bindschädler, A. Adibekian, T. Adam and P. H. Seeberger, *Chem. Biol.*, 2011, **18**, 580; (*e*) D. Safari, H. A. T. Dekker, J. A. F. Joosten, D. Michalik, A. C. de Souza, R. Adamo, M. Lahmann, A. Sundgren, S. Oscarson, J. P. Kamerling and H. Snippe, *Infect. Immun.*, 2008, **76**, 4615; (*f*) A. Kabanova, I. Margarit, F. Berti, M. R. Romano, G. Grandi, G. Bensi, E. Chiarot, D. Proietti, E. Swennen, E. Cappelletti, P. Fontani, D. Casini, R. Adamo, V. Pinto, D. Skibinski, S. Capo, G. Buffi, M. Gallotta, W. J. Christ, A. Stewart Campbell, J. Pena, P. H. Seeberger, R. Rappuoli and P. Costantino, *Vaccine*, 2010, **29**, 104; (*g*) T. J. Boltje, W. Zhong, J. Park, M. A. Wolfert, W. X. Chen and G. J. Boons, *J. Am. Chem. Soc.*, 2012, **134**, 14255; (*h*) Y. L. Huang, J. T. Hung, S. K. C. Cheung, H. Y. Lee, K. C. Chu, S. T. Li, Y. C. Lin, C. T. Ren, T. J. R. Cheng, T. L. Hsu, A. L. Yu, C. Y. Wu and C. H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 2517.

15 (*a*) M. Blaukopf, B. Müller, A. Hofinger and P. Kosma, *Eur. J. Org. Chem.*, 2012, **1**, 119; (*b*) M. A. Oberli, M. Tamborrini, Y. H. Tsai, D. B. Werz, T. Horlacher, A. Adibekian, D. Gauss, H. M. Möller, G. Pluschke and P. H. Seeberger, *J. Am. Chem. Soc.*, 2010, **132**, 10239; (*c*) T. Buskas, Y. Li and G. J. Boons, *Chem. – Eur. J.*, 2004, **10**, 3517; (*d*) D. Safari, M. Marradi, F. Chiodo, H. A. T. Dekker, Y. L. Shan, R. Adamo, S. Oscarson, G. T. Rijkers, M. Lahmann, J. P. Kamerling, S. Penades and H. Snippe, *Nanomedicine*, 2012, **7**, 651; (*e*) T. Horlacher, M. A. Oberli, D. B. Werz, L. Kröck, S. Bufali, R. Mishra, J. Sobek, K. Simons, M. Hirashima, T. Niki and P. H. Seeberger, *ChemBioChem*, 2010, **11**, 1563; (*f*) E. J. Grayson, G. J. L. Bernardes, J. M. Chalker, O. Boutureira, J. R. Koeppe and B. G. Davis, *Angew. Chem., Int. Ed.*, 2011, **50**, 4127.

16 (*a*) D. Crich and L. Li, *J. Org. Chem.*, 2009, **74**, 773; (*b*) P. Bindschädler, C. Noti, E. Castagnetti and P. H. Seeberger, *Helv. Chim. Acta*, 2006, **89**, 2591.

17 (*a*) S. S. Ghosh, P. M. Kao, A. W. Mccue and H. L. Chappelle, *Bioconjugate Chem.*, 1990, **1**, 71; (*b*) F. Wojcik, A. G. O'Brien, S. Götze, P. H. Seeberger and L. Hartmann, *Chem. – Eur. J.*, 2013, **19**, 3090; (*c*) J. Kubler-Kielb, E. Vinogradov, G. Ben-Menachem, V. Pozsgay, J. B. Robbins and R. Schneerson, *Vaccine*, 2008, **26**, 3587.

18 ZPS fragments **1** and **2** were equipped with different linkers in line with our studies on the immunological implications of linker length.

19 K. M. Halkes, D. J. Lefeber, C. T. Fransen, J. P. Kamerling and J. F. Vliegenthart, *Carbohydr. Res.*, 1998, **308**, 329.

20 R. N. Salvatore, R. A. Smith, A. K. Nischwitz and T. Gavin, *Tetrahedron Lett.*, 2005, **46**, 8931.

21 The diastereoselectivities of the depicted glycosylations between **3** and **4** were in the range of 2.1 : 1 to 2.4 : 1 (α : β). Neither additives, such as thiophene (ref. 44*a*) or DMF (ref. 44*b*), nor the use of toluene/dioxane as a solvent mixture (ref. 44*c*) altered this ratio significantly(*a*) S. R. Lu, Y. H. Lai, J. H. Chen, C. Y. Liu and K. K. T. Mong, *Angew. Chem., Int. Ed.*, 2011, **50**, 7315; (*b*) J. Park, S. Kawatkar, J. H. Kim and G. J. Boons, *Org. Lett.*, 2007, **9**, 1959; (*c*) A. V. Demchenko, E. Rousson and G. J. Boons, *Tetrahedron Lett.*, 1999, **40**, 6523.

22 (*a*) J. D. C. Codee, R. E. J. N. Litjens, R. den Heeten, H. S. Overkleeft, J. H. van Boom and G. A. van der Marel, *Org. Lett.*, 2003, **5**, 1519; (*b*) D. Cow and W. J. Li, *Org. Lett.*, 2006, **8**, 959.

23 J. Tatai and P. Fügedi, *Org. Lett.*, 2007, **9**, 4647.

24 G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, 1990, **31**, 1331.

25 W. Zou, J. R. Brisson, S. Larocque, R. L. Gardner and H. J. Jennings, *Carbohydr. Res.*, 1999, **315**, 251.

26 2,4,6-Tri-*tert*-butylpyridine (TTBPy) was employed instead of the more commonly used 2,4,6-tri-*tert*-butylpyrimidine (TTBP) because of the higher basicity of the former reagent.

27 (*a*) P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, 1986, **149**, C9; (*b*) M. Ravenscroft, R. M. G. Roberts and J. G. Tillett, *J. Chem. Soc., Perkin Trans. 2*, 1982, **12**, 1569.

28 (*a*) O. L. Saferni, R. N. Clark and B. E. Smart, *J. Chem. Soc. C*, 1966, 645; (*b*) Alcohol **11** was chosen instead of alcohol **4** for further method evaluation because the respective products were easier to purify by flash chromatography.

29 E. M. Scanlan, M. M. Mackeen, M. R. Wormald and B. G. Davis, *J. Am. Chem. Soc.*, 2010, **132**, 7238.

30 S. Nilsson, H. Lönn and T. Norberg, *Glycoconjugate J.*, 1991, **8**, 9.

31 (*a*) L. J. van den Bos, J. D. C. Codee, R. E. J. N. Litjens, J. Dinkelaar, H. S. Overkleeft and G. A. van der Marel, *Eur. J. Org. Chem.*, 2007, **24**, 3963; (*b*) L. J. van den Bos, J. D. C. Codee, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleeft and G. A. van der Marel, *Org. Lett.*, 2004, **6**, 2165; (*c*) S. Kramer, B. Nolting, A. J. Ott and C. Vogel, *J. Carbohydr. Chem.*, 2000, **19**, 891; (*d*) Y. Nakahara and T. Ogawa, *Nippon Nogei Kagaku Kaishi*, 1988, **62**, 1259; (*e*) A. E. Christina, J. A. Muns, J. Q. A. Olivier, L. Visser, B. Hagen, L. J. van den Bos, H. S. Overkleeft, J. D. C. Codee and G. A. van der Marel, *Eur. J. Org. Chem.*, 2012, **29**, 5729.

32 M. Lahmann and S. Oscarson, *Can. J. Chem.*, 2002, **80**, 889.

33 E. A. Biessen, D. M. Beuting, H. C. Roelen, G. A. van der Marel, J. H. van Boom and T. J. van Berkel, *J. Med. Chem.*, 1995, **38**, 1538.

34 B. Fraser-Reid, Z. F. Wu, U. E. Udodong and H. Ottosson, *J. Org. Chem.*, 1990, **55**, 6068.

35 R. Pragani, P. Stallforth and P. H. Seeberger, *Org. Lett.*, 2010, **12**, 1624.

36 (*a*) P. A. Illarionov, V. I. Torgov and V. N. Shibaev, *Russ. Chem. Bull.*, 2000, **49**, 1895; (*b*) P. A. Illarionov, V. I. Torgov, I. C. Hancock and V. N. Shibaev, *Tetrahedron Lett.*, 1999, **40**, 4247.

37 (*a*) A. E. Christina, V. M. B. Ferrando, F. de Bordes, W. A. Spruit, H. S. Overkleeft, J. D. C. Codee and G. A. van der Marel, *Carbohydr. Res.*, 2012, **356**, 282; (*b*) C. M. Pedersen, I. Figueroa-Perez, J. Boruwa, B. Lindner, A. J. Ulmer, U. Z. ähringer and R. R. Schmidt, *Chem. – Eur. J.*, 2010, **16**, 12627.

38 A. Marinier, A. Martel, C. Bachand, S. Plamondon, B. Turmel, J. P. Daris, J. Banville, P. Lapointe, C. Ouellet, P. Dextraze, M. Menard, J. J. Wright, J. Alford, D. Lee, P. Stanley, X. Nair, G. Todderud and K. M. Tramposch, *Bioorg. Med. Chem.*, 2001, **9**, 1395.

39 (*a*) P. J. Garegg, *Acc. Chem. Res.*, 1992, **25**, 575; (*b*) M. Ohlin, R. Johnsson and U. Ellervik, *Carbohydr. Res.*, 2011, **346**, 1358; (*c*) A. A. Sherman, Y. V. Mironov, O. N. Yudina and N. E. Nifantiev, *Carbohydr. Res.*, 2003, **338**, 697; (*d*) J. Harangi, A. Liptak, V. A. Olah and P. Nanasi, *Carbohydr. Res.*, 1981, **98**, 165; (*e*) Z. W. Guo, S. J. Deng and Y. Z. Hui, *J. Carbohydr. Chem.*, 1996, **15**, 965; (*f*) A. Liptak, L. Janossy, J. Imre and P. Nanasi, *Acta Chim. Hung.*, 1979, **101**, 81.

40 Dioxolane-type benzylidene protection of carbohydrates may result in the preferential formation of one diastereomer (see ref. 37*c* and *f*).

41 A. Liptak, P. Fügedi and P. Nanasi, *Tetrahedron*, 1979, **35**, 1111.

42 M. G. Beaver and K. A. Woerpel, *J. Org. Chem.*, 2010, **75**, 1107.

43 (*a*) K. Agoston, J. Kerekgyarto, J. Hajko, G. Batta, D. J. Lefeber, J. P. Kamerling and J. F. G. Vliegenthart,

 This journal is © The Royal Society of Chemistry 2014

Chem. – Eur. J., 2002, **8**, 151; (*b*) M. Ek, P. J. Garegg, H. Hultberg and S. Oscarson, *J. Carbohydr. Chem.*, 1983, **2**, 305.

44 T. Rosen, I. M. Lico and D. T. W. Chu, *J. Org. Chem.*, 1988, **53**, 1580.

45 J. N. Bemiller and G. V. Kumari, *Carbohydr. Res.*, 1972, **25**, 419.

46 Introduction of a *p*-methoxybenzyl (PMB) ether from the corresponding trichloroacetimidate under acidic conditions was also attempted, but was not compatible with the thioether moiety.

47 (*a*) R. Kartika and R. E. Taylor, *Angew. Chem., Int. Ed.*, 2007, **46**, 6874; (*b*) W. Muramatsu, K. Mishiro, Y. Ueda, T. Furuta and T. Kawabata, *Eur. J. Org. Chem.*, 2010, **5**, 827.

48 (*a*) M. Oikawa, T. Tanaka, S. Kusomoto and M. Sasaki, *Tetrahedron Lett.*, 2004, **45**, 787; (*b*) D. Sawada and Y. Ito, *Tetrahedron Lett.*, 2001, **42**, 2501; (*c*) M. Matteucci, *Tetrahedron Lett.*, 1990, **31**, 2385.

49 (*a*) Y. U. Kwon, R. L. Soucy, D. A. Snyder and P. H. Seeberger, *Chem. – Eur. J.*, 2005, **11**, 2493; (*b*) Oligosaccharides were lef at room temperature under air for 16 h after global deprotection to induce disulfide formation. Only traces of free thiol were observed.

50 (*a*) J. L. de Paz and P. H. Seeberger, *Methods Mol. Biol.*, 2012, **808**, 1; (*b*) Y. Liu, A. S. Palma and T. Feizi, *Biol. Chem.*, 2009, **390**, 647.

51 (*a*) J. A. Burns, J. C. Butler, J. Moran and G. M. Whitesides, *J. Org. Chem.*, 1991, **56**, 2648; (*b*) E. B. Getz, M. Xiao, T. Chakrabarty, R. Cooke and P. R. Selvin, *Anal. Biochem.*, 1999, **273**, 73.

52 (*a*) D. J. Scurr, T. Horlacher, M. A. Oberli, D. B. Werz, L. Kröck, S. Bufali, P. H. Seeberger, A. G. Shard and M. R. Alexander, *Langmuir*, 2010, **26**, 17143; (*b*) T. Horlacher, M. A. Oberli, D. B. Werz, L. Kröck, S. Bufali, R. Mishra, J. Sobek, K. Simons, M. Hirashima, T. Niki and P. H. Seeberger, *ChemBioChem*, 2010, **11**, 1563.

53 H. S. Seo, R. T. Cartee, D. G. Pritchard and M. H. Nahm, *J. Bacteriol.*, 2008, **190**, 2379.

54 (*a*) T. Chida, N. Okamura, K. Ohtani, Y. Yoshida, E. Arakawa and H. Watanabe, *Microbiol. Immunol.*, 2000, **44**, 161; (*b*) D. M. Aanensen, A. Mavroidi, S. D. Bentley, P. R. Reeves and B. G. Spratt, *J. Bacteriol.*, 2007, **189**, 7856; (*c*) A. Mavroidi, D. M. Aanensen, D. Godoy, I. C. Skovsted, M. S. Kaltoft, P. R. Reeves, S. D. Bentley and B. G. Spratt, *J. Bacteriol.*, 2007, **189**, 7841; (*d*) M. J. Coyne, A. O. Tzianabos, B. C. Mallory, V. J. Carey, D. L. Kasper and L. E. Comstock, *Infect. Immun.*, 2001, **69**, 4342.