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Recent progress in chemical synthesis of bacterial surface glycans

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Abstract

With the continuing advancement of carbohydrate chemical synthesis, bacterial glycomes have become increasingly attractive and accessible synthetic targets. While bacteria also produce carbohydrate-containing secondary metabolites, our review here will cover recent chemical synthetic efforts on bacterial surface glycans. The obtained compounds are excellent candidates for the development of improved structurally defined glycoconjugate vaccines against bacterial infection. They are also important probes for investigating glycan-protein interactions. Glycosylation strategies applied for the formation of some challenging glycosidic bonds of various uncommon sugars in a number of recently synthesized bacterial surface glycans are highlighted.

Keywords

bacterial glycan; carbohydrate; glycosyltransferase; glycoconjugate vaccine; synthesis

Introduction

Carbohydrates are presented on the surface of all types of cells and cells from different species. In addition to human glycome which has been the focus of synthetic efforts, there is a recent surge on developing efficient synthetic methods to explore structures and functions of bacterial glycomes. Comparing to human glycome which is composed of 9 basic monosaccharide building blocks with additional carbohydrate structural modifications taking place after or before the formation of glycosidic bonds, bacterial glycomes are of a much larger scope with many more building blocks, an extensive list of additional modifications, and much more diverse glycosidic linkages and branches. Therefore, the synthesis of bacterial glycans poses enormous challenges.

Bacterial surface glycans are important molecules. They are either essential for bacterial survival or are commonly components of virulence factors of bacterial pathogens. Among

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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bacterial surface glycans, peptidoglycans, glycoproteins for some species, and capsular polysaccharides (CPSs) are presented by both Gram-positive and Gram-negative bacteria. The former also contain lipoteichoic acids (LTAs) and wall teichoic acids (WTAs) while the latter contain lipopolysaccharides (LPSs) or lipooligosaccharides (LOSs) [1]. Some bacteria also produce exopolysaccharides (EPSs) which are a part of biofilm [2]. Carbohydrate-containing structures of mycobacteria envelop include arabinogalactan conjugated to the peptidoglycan, arabinomannan, phosphatidyl-myo-inositol-mannosides, lipoarabinomannan and other glycolipids [1]. Bacterial surface glycans especially CPSs and LPSs have been attractive targets for the development of glycoconjugate vaccines to combat deadly bacterial infections including those challenged with antibiotic resistant strains [3,4].

Most currently available clinical bacterial polysaccharide and glycoconjugate vaccines use polysaccharides extracted and purified from bacterial cell culture which are heterogeneous and prone to batch-to-batch variation [5]. Downstream chemical functionalization and conjugation processes involved in the production of bacterial polysaccharide-protein conjugate vaccines add additional heterogeneity and challenges for product quality control [6–8]. The design, chemical synthesis, and clinical application of a successful synthetic *H. influenzae* type b (Hib) CPS oligo(ribosylribitol-phosphate)-conjugate vaccine (Quimi-Hib) [9] inspired efforts for synthesizing structurally defined bacterial CPS and LPS oligosaccharides for conjugate vaccine development. The synthetic glycoconjugate vaccines have advantages of providing higher batch-to-batch reproducibility and simplifying quality control processes. In addition, the ability of selecting representing RU frameshift, varying the length, and improving the design of synthetic oligosaccharide targets using a medicinal chemistry approach [10–12], has the potential of developing glycoconjugate vaccines that are more stable, can induce immune responses superior to those using purified polysaccharides with less defined structures, and providing better protections against bacterial infection [13]. Furthermore, synthetic CPS oligosaccharides can be used for generating multivalent synthetic glycoconjugate vaccines or co-formulation with licensed multivalent CPS glycoconjugate vaccines to expand protection as demonstrated recently in animal studies against *Streptococcus pneumoniae* infection [11,12].

Structurally defined synthetic bacterial surface glycans are also indispensable probes to improve our understanding of bacterial glycan-protein interactions. Synthetic microbial glycan microarrays have been developed as powerful tools to elucidate the binding preference of carbohydrate-binding proteins and to identify oligosaccharides with strong binding to antibodies raised using bacterial polysaccharide glycoconjugate vaccines [1,12,14,15]. Structurally defined bacterial glycans can also be biomaterials and be used as important probes for biochemical characterization of the corresponding biosynthetic and metabolic enzymes [16].

Recently synthesized bacterial surface glycans by chemical strategies are presented with highlights on glycosylation strategies applied for the formation of some challenging glycosidic bonds of various uncommon sugars. Bacterium-produced carbohydrate-containing secondary metabolites [17] and production of bacterial polysaccharides by microbial engineering and fermentation [18] are not discussed.

Monosaccharides of the recently synthesized bacterial surface glycans

Since 2017, a large number of structurally diverse bacterial surface glycans containing diverse monosaccharide building blocks ranging from pentose and pentosamine, hexose, hexosamine, hexuronic acid, hexosaminuronic acid, heptose, to octulosonic acid and nonulosonic acids have been successfully obtained by chemical synthesis. It is impossible to capture all exciting achievements made in the field here. The examples presented include 7 common monosaccharides that are also part of the human glycome and 51 monosaccharides and derivatives that are absent from the human glycome (Figure 1).

Chemically synthesized bacterial surface oligosaccharides

Chemical synthesis remains a main approach to obtain structurally defined bacterial oligosaccharides and polysaccharides, especially those containing uncommon sugars. Convergent [10,19–43] and linear synthetic strategies with [12,44,45] or without [46–78], automated glycan assembly (AGA) have been applied for synthesizing complex oligosaccharides including those of relatively large sizes [43,45] and those with branches.

Synthetic targets chemically obtained include repeating units (RUs) and related oligomers or analogs of CPSs (**1–25**) [10,12,19–26,46–59], LPSs and LOSs (**26–49**) [28–37,60–72], cell wall polysaccharides (**50–53**) [38,39,73], EPSs (**54**) [74], lipoteichoic acid (**55**) [40], pilin glycan (**56**) [75], biofilms polysaccharides (**57**) [41] and mycobacterial surface arabinomannan and arabinogalactan oligosaccharides (**58–60**) [42–44] (Figure 2). The list is not exclusive but is a good representation of the diverse structures that have been recently accessed by chemical synthesis.

The CPS oligosaccharides and analogs obtained (Figure 2A) include those from Hib (**1**) [19]; *S. pneumoniae* (Sp) serotypes ST1 (**2**) [20]**, ST2 (**3**) [10], ST3 (**4–5**) [21]**, ST5 (**6**) [46], ST8 (**7a–7d**) [12]**, ST11A (**8**) [22], and ST12F (**9**) [47]; group B *Streptococcus* (GBS) or *Streptococcus agalactiae* types Ia (**10**) [23], II (**11**) [24], VII (**12**) [25]; carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp) (**13**) [26]; *A. baumannii* K47 (**14**) [48]; *S. aureus* types 5 (**15**) [49,61] and strain M (**16**) [50]; *B. mallei* (**17**) [51,52]; *C. jejuni* RM1221 (**18**) [53]** and 81–176 (**19**) [54]; *Bacteroides fragilis* CPS PS A1 RU (**20**) [55]; *Morganella morganii* (**21**) [56]; *C. bolteae* (**22**) [27]**, *K. kingae* KK01 (**23**) [57], *C. psychrerythraea* 34H (**24**) [58] and *S. typhi* (**25**) [59]. Among these, compounds **2**, **20**, **21**, and **45** are repeating units of zwitterionic polysaccharides (ZPSs).

The LPS and LOS oligosaccharides and analogs obtained (Figure 2B) include those from *B. pertussis* (**26**) [60]**; *P. aeruginosa* STO11 (**27**) [61]**; *P. shigelloide* ST51 (**28**) [62]; *B. cenocepacia* (**29**) [28]; *P. fluorescens* BIM B-582 (**30**) [63]; *H. pylori* STO2 (**31**) [29]**; *K. pneumoniae* O2a (**32**) [30]**; *P. chloroaphis* UCM B-106 (**33**) [64]; *S. boydii* type type 18 (**34**) [65]; *S. enteritidis* (**35**) [31]; *S. paratyphi* A (**36**) [32]**; *S. enterica* O41 (**37**) [33], O51 (**38**) [66], O60 (**39**) [67]; *E. coli* O43 (**40**) [68], O75 (**41**) [69], O115 (**42**) [34], O156 (**43**) [70]; *A. doebereineriae* type strain GSF71^T (**44**) [71], *P. alcalifaciens* O22 (**45**) [78], rhizobial and agrobacterial LPS inner core (**46**) [72]; and *C. jejuni* LOS core (**47**) [35]**; *Bradyrhizobium* sp. BTAi1 (**48**) [36]** and *P. cryohalolentis* K5^T (**49**) [37].

Other bacterial surface glycan oligosaccharides and analogs have been synthesized (Figure 2C) include those of group A *Streptococcus* (GAS) cell-wall glycans (**50**) [38]; *Bacillus anthracis* secondary cell wall glycan (**51**) [73]**; *E. saburreum* strain T19 cell wall glycan (**52**) [39]; *S. natans* cell wall glycan trisaccharide component (**53**) [76,77]**; *B. multivorans* EPS (**54**) [74]; lipoteichoic acid glycans from *C. difficile* (**55**) [40]; *P. aeruginosa* 1244 pilin (**56**) [75]**; *K. pneumoniae* biofilms (**57**) [41]; mycobacterial glycan (**58**) [42], arabinomannan oligosaccharides containing α 1–6-linked Man, α 1–2-linked Man, and/or α 1–5-linked Ara ranging from hexasaccharide to dodecasaccharide (**59**) [44], and a 92-mer arabinogalactan **60** [43]**.

Highlights of glycosylation strategies for constructing challenging linkages in chemical synthesis of bacterial surface glycans

The challenges of chemically installing glycosidic linkages in a stereo- and/or regio-selective manner with high efficiency are mitigated by designing effective orthogonally protected building blocks of varied reactivities with controllable stereo- and/or regio-selectivities [79,80]; choosing suitable glycosylation methods and optimizing reaction conditions; and applying post-glycosylational modification processes (epimerization, oxidation, reduction etc.). De novo synthesis and streamlined preparation processes have been developed for some of the rare sugar building blocks. Some protecting groups have shown power in directing more challenging 1,2-*cis*-glycosylation reactions. One-pot sequential glycosylation [81] and automated glycan assembly (AGA) [16,82] strategies have seen increased applications in the synthesis of bacterial glycans which simplify the synthetic procedures when reaction routes are carefully designed.

Due to the large number of synthetic bacterial surface glycans that have been reported since 2017 and the space limitation here, only some of the examples for the formation of the following challenging glycosylation linkages are discussed briefly below. These include β -L-rhamnose (β -L-Rha) (Figure 3A), β -D-Rha, α/β -L-FucNAc (Figure 3B), β -ManNAcA (Figure 3C), heptose (Figure 3D), α/β -Kdo (Figure 3E), α/β -Pseudaminic acid (Pse) (Figure 3F), and furanosides (Figure 3G). The emphasis is on the strategies used in glycosidic bond formation without detailed discussion on proper designing and installation of orthogonally protected donors and acceptors with desired reactivity.

L-Rha is not found in human glycome but is abundant in plants. Both α - and β -linked L-Rha has also been found in bacterial surface glycans. β -L-Rha (1,2-*cis*) linkage is difficult to form stereoselectively. In addition to unfavored by neighboring group participation, unfavored anomeric effect, steric hindrance of group at C-2, and the lack of C6-OH to install suitable protecting groups for further control of stereochemistry all contribute to the challenges [83]. Several strategies have been developed to overcome the problem. For example, a remote C3-*O*-picoloyl group in a rhamnosyl thioglycoside donor (**F3A_1**) was used to install the 1,2-*cis*-glycosyl linkage in the L-Rha- β 1–4Glc disaccharide building block (**F3A_3**) of compound **3** [10] stereoselectively (53% yield with an $\alpha:\beta$ ratio of 1:3) by a hydrogen-bond-mediated aglycon delivery (HAD) strategy (Figure 3A). A similar strategy was used for synthesizing the L-Rha- β 1–4GlcNAc disaccharide building block (**F3A_6**) of compound **30** with 80% yield and an $\alpha:\beta$ ratio of 1:8.4 [63]. L-Rha- β 1–4-L-Rha and L-Rha-

β 1–4GlcNAc disaccharide units in compounds **34** [65] and **42** [34], respectively, were obtained similarly. Another strategy is to synthesize a C2'-epimer of L-Rha- β 1–4GlcNAc disaccharide building block (**F3A_9**) by an easier to control 1,2-*trans*-glycosylation of an L-quinovose donor (a C2'-epimer of L-Rha building block) (**F3A_7**) followed by post-glycosylational inversion of the C-2 of the L-quinovose in the disaccharide (**F3A_8**) [63]. Recently, 4-*nitro*-substitution of the picoloyl group in the rhamnosyl thioglycoside donor (**F3A_10**) was found to further improve its HAD effect for stereoselective formation (88% yield and an α : β ratio of 1:15) of L-Rha- β 1–4Glc disaccharide building block (**F3A_12**) of compound **53** [76]**. Additionally, a 1,2-anhydro-L-rhamnopyranose donor (**F3A_13**) and a boronic ester formed from a Glc diol acceptor (**F3A_14**) and 4-nitrophenylboronic acid were used to form a similar disaccharide (**F3A_15**) of compound **53** in 88% yield with a complete stereospecific control and high regio-selectivity [77]**.

Compared to L-Rha, D-Rha is less common and the corresponding monosaccharide building blocks are prepared from readily available monosaccharides (e.g. Glc or Man). Formation of β -D-Rha (1,2-*cis*) linkage poses similar challenges as for β -L-Rha. Several strategies were explored for the formation of β -D-Rha in synthesizing *C. bolteae* CPS oligosaccharides **22** [27]**. Success was achieved using indirect methods by either formation of β -D-mannose linkage followed by deoxygenation of C6-OH of the mannose building block or 1,2-*trans*-glycosylation of quinovose followed by oxidation and reduction of the C2-OH of the quinovose building block. A high-yield programmed synthesis using the obtained disaccharide building block led to the construction of oligosaccharides up to an octadecasacchride [27]**.

L-FucNAc is a rare sugar found frequently in bacterial polysaccharides. Its 2-azido-analogs (**F3B_1** and **F3B_4**) were synthesized from L-fucose via a fucal intermediate [46,47,62] or from inexpensive L-Rha by inversion of *O*-triflates at both C2 and C4 [60,61]. Its 2-*N*-Troc-analog (**F3B_12**) was synthesized from L-Rha via a 3-*O*-trichloroacetimidate protected β -rhamnoside intermediate (**F3B_10**) by *in situ* inversion of C4 after its hydroxyl was triflated [60]**.

The 1,2-*cis*-fucosamine linkage in L-FucNAc- α 1–3-D-FucNAc disaccharide building block (**F3B_3**) of compound **15** [49] was installed as a single anomer in 76% yield from a selenoglycoside donor **F3B_1** where electron-donating *tert*-butyldimethylsilyl protecting groups favor the formation of the α -glycosidic bond. Recently, assembling of a similar disaccharide (**F3B_6**) of **15** was achieved stereoselectively from a thioglycoside donor **F3B_4** in 94% yield as a single isomer [61]** where the OBz group at C4 may contribute favorable stereoelectronic effect for the high stereoselectivity observed. A fucosyl trichloroacetimidate donor (**F3B_7**) was also used successfully used for stereospecific formation (77% yield, α -isomer only) of the L-FucNAc- α 1–3-GalNAc linkage in the pentasaccharide building block **F3B_9** of compound **9** by TMSOTf activation with a remote participation of the 3-*O*-acetate group in the donor [47]. A similar strategy was used for the formation of L-FucNAc- α 1–3-D-FucNAc and L-FucNAc- α 1–3/4-QuiNAc linkages in compounds **6** [1], **27** [61]**, and **28** [62] respectively.

The β -L-fucosamine linkage in the trisaccharide building block (**F3B_14**) of compound **26** was formed stereoselectively using the 2-NHTroc thioglycoside donor (**F3B_12**) with a

disaccharide acceptor (**F3B_13**) in the presence of *p*-TolSCI/AgOTf. NIS/TfOH promoter was found unsuitable which may be due to the neutralization of the secondary amine in the donor by TfOH [60]**.

β -ManNAcA is a component of compounds **9**, **15**, and **26**. To introduce ManNAcA, its 2-azido-type donors are commonly synthesized from Glc. The desired β -ManN₃A linkage can be formed directly with the β -directing effect of the C6-carboxyl ester such as in the case of the formation of a trisaccharide building block (**F3C_3**) of compound **15** using an *N*-phenyltrifluoroacetimidate glycosyl donor (**F3C_1**) with a 75% yield and an excellent β -specificity. The use of an excess amount of the donor and a similar amount of a Lewis acid promotor was the most effective for this glycosylation [49]. Alternatively, an indirect approach with post-glycosylational epimerization and oxidation steps was applied such as the case for the formation of a trisaccharide building block (**F3C_9**) of compound **15** [61]** and the ManNAcA- β 1-3-L-FucNAc linkage in compounds **26** [60]**. For example for the synthesis of **F3C_9**, a Glc donor (**F3C_4**) was coupled with disaccharide **F3C_5** with NIS and TMSOTf promoter leading to the formation of 1,2-*trans*-linked glucosyl trisaccharide **F3C_6** which was converted to the desired ManNAcA- β 1-3-L-FucNAc component by epimerization of the C2 of the glucose followed by oxidation of C6 [61]**. The β -ManNAcA linkage in compound **9** was formed using a 3-*O*-TBS and 4,6-benzylidene-protected 2-N₃ mannosyl thioglycoside donor for glycosylation followed by late-stage oxidation [47].

Bacterial glycans **17**, **18**, **19**, **26**, **52**, and **47** contain one or more rare heptose residues. Heptose building blocks were obtained by de novo synthesis via aldol reactions or by carbon chain extension of hexoses. For example, de novo synthesis of a 6-deoxy-D-manno-heptose building block **F3D_4** was achieved from D-proline-catalyzed aldol reaction of 3-carbon ketone **F3D_1** and 2,2-dimethoxyacetaldehyde **F3D_2** followed by an LDA-promoted aldol reaction with benzyloxyacetaldehyde. *O*-Hexynylbenzoate glycosyl donor **F3D_5** was designed and applied for gold(I) complex-catalyzed formation of the β -linkage in a disaccharide building block (**F3D_7**) of compound **18** [53]**. On the other hand, 6-deoxy-manno-heptose **F3D_10** was prepared from D-mannose via a thiomannoside intermediate (**F3D_8**) which was oxidized at C6 followed by a Wittig reaction, hydroboration of the resulting olefin with 9-borabicyclo[3.3.1]nonane (9-BBN), and an oxidative workup. Installation of 3-*O*-picoloyl group in the glycosyl donor **F3D_12** allowed stereoselective glycosylation with NIS/TfOH promoter to produce a β -linked disaccharide building block **F3D_14** of compound **17** in high yield (85%) via a remote HAD strategy [51]. An L-glycero-D-manno-heptose thioglycoside donor was prepared from a mannose thioglycoside similarly via oxidation, Wittig olefination, and dihydroxylation by osmium tetroxide for the synthesis of **26** [60]**. Furthermore, an L-glycero-D-manno-heptose building block (**F3D_18**) was synthesized from a mannoside (**F3D_15**) via a Grignard reaction with vinyl-magnesium bromide followed by Lemieux-Johnson oxidation and then reduction for the synthesis of **47** [35]**.

3-Deoxy-D-manno-oct-2-ulosonic acid (Kdo) is a bacterium-specific α -keto acid with an eight carbon backbone. It is a characteristic component of bacterial LPSs and CPSs where

both α (axial)- and β (equatorial)-linkages have been found. An α -Kdo glycoside (**F3E_5**) for producing **47** was stereoselectively synthesized from commercially available 2,3:5,6-di-*O*-isopropylidene- α -D-mannofuranose (**F3E_1**) via an 2,3-ene Kdo intermediate (**F3E_2**) using an electrophilic phenylselenenyl trifluoromethanesulfonate (PhSeOTf) as a promoter [35]**. The α -Kdo linkage in the core oligosaccharide (**46**) was achieved with a 4,5;7,8-di-*O*-isopropylidene Kdo glycosyl fluoride donor [72]. On the other hand, a Kdo *ortho*-hexynylbenzoate donor **F3E_8** was synthesized from readily available D-arabinose and oxalacetic acid by a decarboxylative aldol reaction. It was used to form a β -Kdo glycoside **F3E_9** of **23** using a gold(I) complex (PPh₃AuOTf) catalyst [57,84]. Furthermore, C5-functionality of Kdo thioglycoside donors and the reactivity and/or steric hindrance of the acceptors were found to influence the stereochemistry outcome of the products. An *O*-arylcarbonyl or *O*-acetyl at C-5 in the donor preferred α -linked products. An *O*-Quin at C-5 preferred β -linked products with primary glycosyl acceptors while α -linked products formed with disarmed or crowded secondary glycosyl acceptors [85]. The conformation of the side chain of Kdo donors also influence the stereochemistry of the products which can be designed to form either α or β -linked products [86].

Pseudaminic acid (Pse) is a bacterial α -keto acid with a nine carbon backbone (nonulosonic acid) that has been identified from surface glycans and glycoproteins of several bacteria with either α (axial)- or β (equatorial)-linkage. It is not readily available and needs to be constructed for synthesizing Pse-containing glycans (Figure 3F). By adopting a *de novo* approach, a 5-*N*-Troc and 7-*N*-Cbz-protected Pse thioglycoside donor (**F3F_9**) was synthesized from L-threonine (**F3F_1**) via an L-*allo*-threonine intermediate (**F3F_2**) by an aldol-type addition with glycine thioester isonitrile (**F3F_4**) and an indium-mediated Barbier allylation reaction with isopropyl bromomethacrylate (**F3F_8**). The Pse donor (**F3F_9**) showed excellent axial selectivity and was used with a disaccharide acceptor (**F3F_10**) for the synthesis of trisaccharide (**F3F_11**) of **56** with 67% yield and an excellent α -selectivity [75]. A 5,7-diazido Pse adamantanyl (Ada) thioglycoside donor (**F3F_12**) was synthesized from Neu5Ac and predicted to have a *trans,gauche* conformation on the side chain. The donor was confirmed experimentally to have an excellent equatorial stereoselectivity for synthesizing β -linked Pse-glycosides such as **F3F_14** [87]**. More recently, new Pse thioglycoside donors bearing an azido group at C5 and an *N*-Cbz group at C7 (**F3F_15**) were synthesized from L-*allo*-threonine. The stereoselectivity of the glycosylation with NIS and TfOH as the promoter can be controlled by changing the solvent. While β -glycosides were the major products when DCM/CH₃CN co-solvent was used, α -pseudaminosides were the major products when DMF in DCM (5.0 equiv) was used. Glycosylation with a galactofuranoside acceptor (**F3F_16**) under the former condition formed a disaccharide building block with a β -Pse linkage (**F3F_17**) for synthesizing a protected trisaccharide **33** [64]**.

Monosaccharide furanose forms are found in many microbial glycoconjugates, such as Gal f in **32** and **58**, Fuc f in **52**, Ara f in **59** and **60**. Compound **52** [39] contains two adjacent 1,2-*cis*-fucofuranosides. The 1,2-*cis*-glycosyl linkage in its D-Fuc f - α 1-4Galhepp disaccharide building block (**F3G_3**) was formed stereoselectively in 92% yield with an α : β ratio of 7:1 using a D-Fuc f donor (**F3G_1**) with the remote participation effect of its C3-*O*-benzoyl

group. Introduction of the second α -D-Fucf was achieved using a 5-*O*-2-quinolinecarbonyl (Quin)-protected donor **F3G_5** via the HAD strategy to obtain the trisaccharide building block **F3G_6** for compound **52** [39]. Construction of the trisaccharide building block **F3G_11** for compound **56** [42] was achieved by glycosylation of acceptor **F3G_8** using a glycosyl fluoride donor **F3G_7** to form disaccharide **F3G_9** which reacted further with disaccharide **F3G_10** to produce tetrasaccharide in an excellent 94% yield.

Automation of chemical synthesis of bacterial surface glycans

Various automation systems have been developed for chemical synthesis of carbohydrates and have been reviewed recently [16,81,88]. It is expected that they will be increasingly applied in synthesizing a growing number of bacterial surface glycans. Additional efforts are needed to further improve the yields and stereoselectivity of glycosylation reactions for some challenging glycosidic linkages discussed above to allow their efficient application in automation. Although post-glycosylation strategies are highly valuable strategies for the formation of some otherwise challenging glycosidic bonds in relatively shorter-chain targets, they could be problematic for synthesizing longer-chain structures by automated processes. Using oligosaccharide building blocks with pre-formed more challenging linkages provides one of the solutions. A broader application of automation for bacterial surface glycan assembly will be further facilitated by increasing the accessibility of building blocks with decreased cost which can be driven by improving methodologies, growing interest in exploring important roles of glycans and rising demands [11] by the broad community.

Applications of the chemically synthesized bacterial surface glycans

Glycan microarray studies of synthetic bacterial surface glycans and glycoconjugate vaccine tests in animal models showed that short-chain oligosaccharides (e.g. Hib CPS oligosaccharides **1** [19], SpST3 CPS oligosaccharides **4–5** [21]**, *C. bolteae* CPS oligosaccharides **22** [27]**) may be more efficient in inducing immune responses than those of larger sizes. Bacterial uncommon sugars and unique glycosyl linkages may be essential recognition component for antibodies [46] and could be part of effective vaccine candidates [12]. Unique monosaccharide modifications, such as 4,6-*O*-pyruvyl ketal-modified ManNAc component of a *Bacillus anthracis* secondary cell wall glycan RU derivative (**51**) [73]**, could be crucial for protein recognition and binding. The ability of accessing all frameshifts of oligosaccharide repeating units such as the case for SpST8 tetrasaccharide repeats (**7a–7d**) [12] in a medicinal chemistry-like approach allowed the identification of the most effective glycoconjugate vaccine candidate. All of these examples showcase the importance and advantages of developing synthetic bacterial glycan-based probes and conjugate vaccines.

Conclusions and perspectives

Chemically synthesized bacterial surface glycans have demonstrated potentials in glycan-protein interaction studies and glycoconjugate vaccine development. Significant synthetic efforts have been devoted with a tremendous degree of success in obtaining a growing number of structurally defined bacterial surface glycans with structural complexity including those containing uncommon monosaccharide units with various modifications, charges,

challenging glycosidic linkages, branches, and large sizes. Some of the challenges in 1,2-*cis*-selective glycosylation have been mitigated by a powerful remote hydrogen-bond-mediated aglycon delivery (HAD) strategy and various mediation groups such as picoloyl (Pico), 4-nitropicoloyl, and 2-quinolinecarbonyl (Quin) have been developed. Streamlined processes have been developed for constructing some of uncommon monosaccharide building blocks (e.g. furanoses, heptoses, BacAc₂, L-FucNAc, L-FucNAc4NH₂ [89]) but the processes can be further improved and much effort is still needed for others (e.g. Pse). Direct glycosylation for the formation of β -D-Rha and β -D-ManNAcA-linkages and effective construction of β -L-FucNAc-linkage stereoselectively await further method development. Automation [16,82,88] with [81] or without one-pot strategy can further speed up the synthesis. The access to a good collection of synthetic bacterial surface glycans by the broad community is still limited or lacking. Joining synthetic efforts with functional studies such as synthetic bacterial glycan microarrays [14,90] and multiplex systems with glycan-immobilized fluorescent beads [91,92] assay platforms, in vitro biochemical and cellular studies, and in vivo animal studies will further advance our understanding of this important class of structurally diverse biomolecules and help to develop effective diagnostics and therapeutics.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:
** of outstanding interest

References

1. Campanero-Rhodes MA, Palma AS, Menendez M, Solis D: Microarray strategies for exploring bacterial surface glycans and their interactions with glycan-binding proteins. *Front Microbiol* 2019, 10:2909. [PubMed: 32010066]
2. Trunk T, Khalil HS, Leo JC: Bacterial autoaggregation. *AIMS Microbiol* 2018, 4:140–164 [PubMed: 31294207]
3. Berti F, Adamo R: Antimicrobial glycoconjugate vaccines: an overview of classic and modern approaches for protein modification. *Chem Soc Rev* 2018, 47:9015–9025. [PubMed: 30277489]
4. Rappuoli R: Glycoconjugate vaccines: Principles and mechanisms. *Sci Transl Med* 2018, 10:eaat4615. [PubMed: 30158151]
5. Colombo C, Pitirollo O, Lay L: Recent advances in the synthesis of glycoconjugates for vaccine development. *Molecules* 2018, 23:1712.
6. Frasch CE: Preparation of bacterial polysaccharide-protein conjugates: analytical and manufacturing challenges. *Vaccine* 2009, 27:6468–6470. [PubMed: 19555714]
7. Micoli F, Costantino P, Adamo R: Potential targets for next generation antimicrobial glycoconjugate vaccines. *FEMS Microbiol Rev* 2018, 42:388–423. [PubMed: 29547971]
8. Gening ML, Kurbatova EA, Tsvetkov YE, Nifantiev NE: Development of approaches to a third-generation carbohydrate-conjugate vaccine against *Streptococcus pneumoniae*: the search for optimal oligosaccharide ligands. *Russian Chem Rev* 2015, 84:1100–1113.

9. Verez-Bencomo V, Fernandez-Santana V, Hardy E, Toledo ME, Rodriguez MC, Heynngnezz L, Rodriguez A, Baly A, Herrera L, Izquierdo M, et al.: A synthetic conjugate polysaccharide vaccine against *Haemophilus influenzae* type b. *Science* 2004, 305:522–525. [PubMed: 15273395]
10. Emmadi M, Khan N, Lykke L, Reppe K, S GP, Lisboa MP, Wienhold SM, Witzenrath M, Pereira CL, Seeberger PH: A *Streptococcus pneumoniae* type 2 oligosaccharide glycoconjugate elicits opsonic antibodies and is protective in an animal model of invasive pneumococcal disease. *J Am Chem Soc* 2017, 139:14783–14791. [PubMed: 28945368]
11. Kaplonek P, Khan N, Reppe K, Schumann B, Emmadi M, Lisboa MP, Xu FF, Calow ADJ, Parameswarappa SG, Witzenrath M, et al.: Improving vaccines against *Streptococcus pneumoniae* using synthetic glycans. *Proc Natl Acad Sci U S A* 2018, 115:13353–13358. [PubMed: 30530654]
12. Schumann B, Hahm HS, Parameswarappa SG, Reppe K, Wahlbrink A, Govindan S, Kaplonek P, Pirofski LA, Witzenrath M, Anish C, et al.: A semisynthetic *Streptococcus pneumoniae* serotype 8 glycoconjugate vaccine. *Sci Transl Med* 2017, 9:eaaf5347. [PubMed: 28275152] **A medicinal chemistry strategy of synthesizing all four frameshifts of SpST8 CPS tetrasaccharide repeat by automated glycan assembly. Glycan microarray study confirmed frameshift 7c was preferentially recognized by CPS-directed mAb, highlighting the advantages of synthetic glycans for vaccine development.
13. Adamo R: Advancing homogeneous antimicrobial glycoconjugate vaccines. *Acc Chem Res* 2017, 50:1270–1279. [PubMed: 28463499]
14. Geissner A, Reinhardt A, Rademacher C, Johannssen T, Monteiro J, Lepenies B, Thepaut M, Fieschi F, Mrazkova J, Wimmerova M, et al.: Microbe-focused glycan array screening platform. *Proc Natl Acad Sci U S A* 2019, 116:1958–1967. [PubMed: 30670663]
15. Zheng RB, Jegouzo SAF, Joe M, Bai Y, Tran HA, Shen K, Saupe J, Xia L, Ahmed MF, Liu YH, et al.: Insights into Interactions of mycobacteria with the host innate immune system from a novel array of synthetic mycobacterial glycans. *ACS Chem Biol* 2017, 12:2990–3002. [PubMed: 29048873]
16. Pardo-Vargas A, Delbianco M, Seeberger PH: Automated glycan assembly as an enabling technology. *Curr Opin Chem Biol* 2018, 46:48–55. [PubMed: 29715619]
17. Elshahawi SI, Shaaban KA, Kharel MK, Thorson JS: A comprehensive review of glycosylated bacterial natural products. *Chem Soc Rev* 2015, 44:7591–7697. [PubMed: 25735878]
18. Moradali MF, Rehm BHA: Bacterial biopolymers: from pathogenesis to advanced materials. *Nat Rev Microbiol* 2020, 18:195–210. [PubMed: 31992873]
19. Baek JY, Geissner A, Rathwell DCK, Meierhofer D, Pereira CL, Seeberger PH: A modular synthetic route to size-defined immunogenic *Haemophilus influenzae* b antigens is key to the identification of an octasaccharide lead vaccine candidate. *Chem Sci* 2018, 9:1279–1288. [PubMed: 29675174]
20. Zhang Q, Gimeno A, Santana D, Wang Z, Valdes-Balbin Y, Rodriguez-Noda LM, Hansen T, Kong L, Shen M, Overkleeft HS, et al.: Synthetic, zwitterionic Sp1 oligosaccharides adopt a helical structure crucial for antibody interaction. *ACS Cent Sci* 2019, 5:1407–1416. [PubMed: 31482123] **Assembly of SpST1 CPS oligosaccharides up to dodecasaccharide containing 4 synthetically challenging zwitterionic trisaccharide RU was achieved. Multiple carboxylate groups were introduced after constructing the repeating unit through a modified two-step one-pot TEMPO/BAIB Pinnick oxidation protocol.
21. Xiong C, Feng S, Qiao Y, Guo Z, Gu G: Synthesis and immunological studies of oligosaccharides that consist of the repeating unit of *Streptococcus pneumoniae* serotype 3 capsular polysaccharide. *Chemistry* 2018, 24:8205–8216. [PubMed: 29528515] **S. pneumoniae type 3 CPS up to octasaccharides were synthesized by a highly convergent strategy and postglycosylation oxidation process was used to generate desired glucuronic acid residues. Glycoconjugates with penta- or hexasaccharides were shown to induce stronger immune responses than those with hepta- or octasaccharides.
22. Si A, Misra AK: Concise synthesis of the phosphoglycerylated tetrasaccharide repeating unit of the capsular polysaccharide of *Streptococcus pneumoniae* serotype 11 A. *ChemistrySelect* 2017, 2:11771–11774.

23. Liao G, Guo J, Yang D, Zhou Z, Liu Z, Guo Z: Synthesis of a dimer of the repeating unit of type Ia group B Streptococcus extracellular capsular polysaccharide and immunological evaluations of related protein conjugates. *Organic Chemistry Frontiers* 2019, 6:2833–2838. [PubMed: 32274071]
24. Shao L, Zhang H, Li Y, Gu G, Cai F, Guo Z, Gao J: Chemical synthesis of the repeating unit of type II group B Streptococcus capsular polysaccharide. *J Org Chem* 2018, 83:5920–5930. [PubMed: 29709182]
25. Zhang H, Shao L, Wang X, Zhang Y, Guo Z, Gao J: One-pot synthesis of the repeating unit of type VII group B Streptococcus polysaccharide and the dimer. *Org Lett* 2019, 21:2374–2377. [PubMed: 30888190]
26. Seeberger PH, Pereira CL, Khan N, Xiao G, Diago-Navarro E, Reppe K, Opitz B, Fries BC, Wittenrath M: A semi-synthetic glycoconjugate vaccine candidate for carbapenem-resistant *Klebsiella pneumoniae*. *Angew Chem Int Ed Engl* 2017, 56:13973–13978. [PubMed: 28815890]
27. Yin J, Cai J, Hu J, Qin C, Li L, Shen D, Tian G, Zou X, Seeberger PH: Chemical synthesis elucidates the key antigenic epitope of the autism-related bacterium *Clostridium botulinum* capsular octadecasaccharide. *Angew Chem, Int Ed* 2020 DOI:10.1002/anie.202007209**Different strategies were compared for the synthesis of challenging 1,2-cis-linked D-Rha. Success was achieved using indirect methods. A high-yield programed synthesis using the obtained disaccharide building block led to the construction of oligosaccharides up to an octadecasaccharide. Tetrasaccharide was found to be a potentially better candidate than longer oligosaccharides for glycoconjugate vaccine development.
28. Bi N, Xiong C, Jin G, Guo Z, Gu G: Synthesis of a trisaccharide repeating unit of the O-antigen from *Burkholderia cenocepacia* and its dimer. *Carbohydr Res* 2017, 451:1–11. [PubMed: 28917967]
29. Tian G, Qin C, Liu Z, Shen D, Zou X, Fu J, Hu J, Seeberger PH, Yin J: Total synthesis of the *Helicobacter pylori* serotype O2 O-antigen α -(1→2)- and α -(1→3)-linked oligoglucosides. *Chem Commun* 2020, 56:344–347.**Stereoselectivity of α -glucosylation in α 1–2- and α 1–3-linked H. pylori O2 O-antigen oligoglucosides was improved using remote acyl group participation, which can be extended for the synthesis of other sterically crowded 1,2-cis-glycosides.
30. Sweeney RP, Lowary TL: A route to polyprenol pyrophosphate-based probes of O-polysaccharide biosynthesis in *Klebsiella pneumoniae* O2a. *Org Lett* 2019, 21:1050–1053. [PubMed: 30681873] **Convergent synthesis K. pneumoniae serotype O2a nonasaccharide was achieved which was further functionalized with a polyprenol pyrophosphate motif. The glycosylation approaches were highly diastereoselective for both 1,2-trans- β and 1,2-cis- α linkages.
31. Huo CX, Dhara D, Baliban SM, Tahmasebi Nick S, Tan Z, Simon R, Misra AK, Huang X: Synthetic and immunological studies of *Salmonella enteritidis* O-antigen tetrasaccharides as potential anti-*Salmonella* vaccines. *Chem Commun* 2019, 55:4519–4522.
32. Dhara D, Baliban SM, Huo CX, Rashidjahanabad Z, Sears KT, Nick ST, Misra AK, Tennant SM, Huang X: Syntheses of *Salmonella paratyphi* A associated oligosaccharide antigens and development towards anti-paratyphoid fever vaccines. *Chem Eur J* 2020, DOI:10.1002/chem.202002401**An indirect approach was developed for 1,2-cis-glycosylation for the formation of an α -paratose linkage in an S. paratyphi A surface glycan. This was achieved by α -glucosylation followed by deoxygenation via reduction of the thionoformate functionality at C-3 and C-6 of the glucose component. Glycoconjugate with bacteriophage Q β induced IgG antibodies against S. paratyphi A in animal models.
33. Bhaumik I, Misra AK: Convergent synthesis of the tetrasaccharide repeating unit of the O-polysaccharide of *Salmonella enterica* O41. *ChemistrySelect* 2017, 2:3065–3067.
34. Kundu M, Guchait A, Misra AK: Convergent synthesis of a pentasaccharide corresponding to the cell wall O-polysaccharide of enteropathogenic *Escherichia coli* O115. *Tetrahedron* 2020, 76:130952.
35. Yoshida F, Yoshinaka H, Tanaka H, Hanashima S, Yamaguchi Y, Ishihara M, Saburomaru M, Kato Y, Saito R, Ando H, et al.: Synthesis of the core oligosaccharides of lipooligosaccharides from *Campylobacter jejuni*: A putative cause of Guillain-Barre Syndrome. *Chemistry* 2019, 25:796–805. [PubMed: 30351481] **Complex octasaccharide and nonasaccharide containing one or two

α -linked Neu5Ac, an α -Kdo, and two α -L-glycero-D-manno-heptoses were synthesized efficiently via a convergent approach with detailed NMR characterization.

36. Li W, Silipo A, Gersby LB, Newman MA, Molinaro A, Yu B: Synthesis of bradyrhizose oligosaccharides relevant to the Bradyrhizobium O-antigen. *Angew Chem, Int Ed* 2017, 56:2092–2096. **Oligosaccharides of a very unique and rare bicyclic monosaccharide called bradyrhizose with challenging α 1–7-glycosidic linkages were synthesized. NMR experiments and molecular dynamics simulation studies showed that pentasaccharide tends to form a right-handed helix.
37. Emmadi M, Kulkarni SS: Synthesis of rare deoxy amino sugar building blocks enabled the total synthesis of a polysaccharide repeating unit analogue from the LPS of *Psychrobacter cryohalolentis* K5(T). *J Org Chem* 2018, 83:14323–14337. [PubMed: 30388010]
38. Zhao Y, Wang S, Wang G, Li H, Guo Z, Gu G: Synthesis and immunological studies of group A *Streptococcus* cell-wall oligosaccharide–streptococcal C5a peptidase conjugates as bivalent vaccines. *Org Chem Front* 2019, 6:3589–3596.
39. Pan X-L, Huang L, Zeng Y, Xu C-Y, Liu D-M, Chu Y, Qin Y, Yang J-S: Synthesis of an unusual hexasaccharide repeating unit from the cell wall polysaccharide of *Eubacterium saburreum* strain T19. *Org Chem Front* 2020, 7:2298–2306.
40. Yu K, Bi N, Xiong C, Cai S, Long Z, Guo Z, Gu G: Synthesis of defined and functionalized glycans of lipoteichoic acid: A cell surface polysaccharide from *Clostridium difficile*. *Org Lett* 2017, 19:3123–3126. [PubMed: 28548838]
41. Guchhait A, Ghosh A, Misra AK: Convergent synthesis of the pentasaccharide repeating unit of the biofilms produced by *Klebsiella pneumoniae*. *Beilstein J Org Chem* 2019, 15:431–436. [PubMed: 30873227]
42. Xue X, Zheng RB, Koizumi A, Han L, Klassen JS, Lowary TL: Synthetic polyprenolpyrophosphate linked oligosaccharides are efficient substrates for mycobacterial galactan biosynthetic enzymes. *Org Biomol Chem* 2018, 16:1939–1957. [PubMed: 29492483]
43. Wu Y, Xiong DC, Chen SC, Wang YS, Ye XS: Total synthesis of mycobacterial arabinogalactan containing 92 monosaccharide units. *Nat Commun* 2017, 8:14851–14857. [PubMed: 28300074]
**Total chemical synthesis of a 92-mer polysaccharide was achieved. The synthetic strategy included preactivation-based one-pot glycosylation synthesis of a number of linear and branched oligosaccharide fragments ranging from 5-mer to 31-mer in length which was used to assemble the final product via a [31+31+30] convergent glycosylation reaction.
44. Pardo-Vargas A, Bharate P, Delbianco M, Seeberger PH: Automated glycan assembly of arabinomannan oligosaccharides from *Mycobacterium tuberculosis*. *Beilstein J Org Chem* 2019, 15:2936–2940. [PubMed: 31839840]
45. Joseph AA, Pardo-Vargas A, Seeberger PH: Total synthesis of polysaccharides by automated glycan assembly. *J Am Chem Soc* 2020, 142:8561–8564. [PubMed: 32338884] **A milestone achievement of automated carbohydrate synthesis of structurally defined α 1–6-mannan of 100 monosaccharide residues with an average of 98.75% yield for individual glycosylation reaction. Convergent coupling of oligosaccharide building blocks obtained by automated synthesis led to the formation of a polymer of 151 monosaccharide residues.
46. Lisboa MP, Khan N, Martin C, Xu FF, Reppe K, Geissner A, Govindan S, Wittenrath M, Pereira CL, Seeberger PH: Semisynthetic glycoconjugate vaccine candidate against *Streptococcus pneumoniae* serotype 5. *Proc Natl Acad Sci U S A* 2017, 114:11063–11068. [PubMed: 28973947]
47. Seeberger PH, Pereira CL, Govindan S: Total synthesis of a *Streptococcus pneumoniae* serotype 12F CPS repeating unit hexasaccharide. *Beilstein J Org Chem* 2017, 13:164–173. [PubMed: 28228857]
48. Zhang Y, Zhang H, Zhao Y, Guo Z, Gao J: Efficient strategy for α -selective glycosidation of D-glucosamine and its application to the synthesis of a bacterial capsular polysaccharide repeating unit containing multiple α -linked GlcNAc residues. *Org Lett* 2020, 22:1520–1524. [PubMed: 32030988]
49. Hagen B, Ali S, Overkleeft HS, van der Marel GA, Codee JD: Mapping the reactivity and selectivity of 2-azidofucosyl donors for the assembly of N-acetylglucosamine-containing bacterial oligosaccharides. *J Org Chem* 2017, 82:848–868. [PubMed: 28051314]

50. Hagen B, van Dijk JHM, Zhang Q, Overkleeft HS, van der Marel GA, Codee JDC: Synthesis of the *Staphylococcus aureus* strain M capsular polysaccharide repeating unit. *Org Lett* 2017, 19:2514–2517. [PubMed: 28485610]
51. Geng X, Wang G, Guo Z, Gu G: Synthesis of the oligosaccharides of *Burkholderia pseudomallei* and *B. mallei* capsular polysaccharide and preliminary immunological studies of their protein conjugates. *J Org Chem* 2020, 85:2369–2384. [PubMed: 31912729]
52. Tamigney Kenfack M, Mazur M, Nualnoi T, Shaffer TL, Ngassimou A, Bleriot Y, Marrot J, Marchetti R, Sintiprungrat K, Chantratita N, et al.: Deciphering minimal antigenic epitopes associated with *Burkholderia pseudomallei* and *Burkholderia mallei* lipopolysaccharide O-antigens. *Nat Commun* 2017, 8:115. [PubMed: 28740137]
53. Wang X, Chen Y, Wang J, Yang Y: Total synthesis of the trisaccharide antigen of the *Campylobacter jejuni* RM1221 capsular polysaccharide via de novo synthesis of the 6-deoxy-D-manno-heptose building blocks. *J Org Chem* 2019, 84:2393–2403. [PubMed: 30691266] **De novo synthesis of a 6-deoxy-D-manno-heptose building block was achieved from D-proline-catalyzed aldol reaction of 3-carbon ketone and 2,2-dimethoxyacetaldehyde followed by an LDA-promoted aldol reaction with benzyloxyacetaldehyde. O-Hexynylbenzoate glycosyl donor was designed and applied for gold(I) complex-catalyzed formation of a challenging β -linkage.
54. Thota VN, Ferguson MJ, Sweeney RP, Lowary TL: Synthesis of the *Campylobacter jejuni* 81–176 strain capsular polysaccharide repeating unit reveals the absolute configuration of its O-methyl phosphoramidate motif. *Angew Chem Int Ed Engl* 2018, 57:15592–15596. [PubMed: 30280458]
55. Eradi P, Ghosh S, Andreana PR: Total synthesis of zwitterionic tetrasaccharide repeating unit from *Bacteroides fragilis* ATCC 25285/NCTC 9343 capsular polysaccharide PS A1 with alternating charges on adjacent monosaccharides. *Org Lett* 2018, 20:4526–4530. [PubMed: 30015493]
56. Keith DJ, Townsend SD: Total synthesis of the congested, bisphosphorylated *Morganella morganii* zwitterionic trisaccharide repeating unit. *J Am Chem Soc* 2019, 141:12939–12945. [PubMed: 31329445]
57. Zhuang L, Chen Y, Lou Q, Yang Y: Synthesis of the beta-linked GalNAc-Kdo disaccharide antigen of the capsular polysaccharide of *Kingella kingae* KK01. *Org Biomol Chem* 2019, 17:1694–1697. [PubMed: 30346002]
58. Vessella G, Casillo A, Fabozzi A, Traboni S, Iadonisi A, Corsaro MM, Bedini E: Synthesis of the tetrasaccharide repeating unit of the cryoprotectant capsular polysaccharide from *Colwellia psychrerythraea* 34H. *Org Biomol Chem* 2019, 17:3129–3140. [PubMed: 30838361]
59. Zhang G-L, Wei M-M, Song C, Ma Y-F, Zheng X-J, Xiong D-C, Ye X-S: Chemical synthesis and biological evaluation of penta- to octa-saccharide fragments of Vi polysaccharide from *Salmonella typhi*. *Org Chem Front* 2018, 5:2179–2188.
60. Wang P, Huo CX, Lang S, Caution K, Nick ST, Dubey P, Deora R, Huang X: Chemical synthesis and immunological evaluation of Pertussis-like pentasaccharide bearing multiple rare sugars as a potential anti-pertussis vaccine. *Angew Chem Int Ed Engl* 2020, 59:6451–6458. [PubMed: 31953912] **A very challenging target with an L-glycero-D-manno-heptose, a β -L-fucosamine and a ManNAcA- β 1–3-L-FucNAc linkages was successfully synthesized and conjugated to bacteriophage Q β which was shown to be an effective carrier for glycoconjugate vaccine development.
61. Behera A, Rai D, Kulkarni SS: Total syntheses of conjugation-ready trisaccharide repeating units of *Pseudomonas aeruginosa* O11 and *Staphylococcus aureus* type 5 capsular polysaccharide for vaccine development. *J Am Chem Soc* 2020, 142:456–467. [PubMed: 31815459] **Streamlined synthesis of FucN3 building blocks from inexpensive L-Rha for synthesizing L-FucNAc-containing oligosaccharides. Challenging 1,2-cis-L-FucNAc and 1,2-cis-ManNAcA were formed stereoselectively.
62. Qin C, Schumann B, Zou X, Pereira CL, Tian G, Hu J, Seeberger PH, Yin J: Total synthesis of a densely functionalized *Plesiomonas shigelloides* serotype 51 aminoglycoside trisaccharide antigen. *J Am Chem Soc* 2018, 140:3120–3127. [PubMed: 29377682]
63. Behera A, Rai D, Kushwaha D, Kulkarni SS: Total synthesis of trisaccharide repeating unit of O-specific polysaccharide of *Pseudomonas fluorescens* BIM B-582. *Org Lett* 2018, 20:5956–5959. [PubMed: 30187759]

64. Wei R, Liu H, Tang AH, Payne RJ, Li X: A solution to chemical pseudaminylation via a bimodal glycosyl donor for highly stereocontrolled α - and β -glycosylation. *Org Lett* 2019, 21:3584–3588. [PubMed: 31045367] **An efficient Pse donor was designed and de novo synthesized to allow stereoselective glycosylation for the formation of either α - or β -Pse linkage with acceptors of different reactivities by changing solvents.
65. Shit P, Gucchait A, Misra AK: Straightforward sequential and one-pot synthesis of a pentasaccharide repeating unit corresponding to the cell wall O-antigen of *Shigella boydii* type 18. *Tetrahedron* 2019, 75:130697.
66. Bhaumik I, Misra AK: Concise synthesis of a pentasaccharide repeating Unit corresponding to the O-antigen of *Salmonella enterica* O51. *ChemistrySelect* 2017, 2:937–939.
67. Gucchait A, Shit P, Misra AK: Concise synthesis of a tetrasaccharide related to the repeating unit of the cell wall O-antigen of *Salmonella enterica* O60. *Tetrahedron* 2020, 76:131412.
68. Shit P, Misra AK: Straightforward synthesis of the pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O43 strain. *Glycoconj J* 2020, 37:647–656. [PubMed: 32601769]
69. Nishi N, Nashida J, Kaji E, Takahashi D, Toshima K: Regio- and stereoselective beta-mannosylation using a boronic acid catalyst and its application in the synthesis of a tetrasaccharide repeating unit of lipopolysaccharide derived from *E. coli* O75. *Chem Commun* 2017, 53:3018–3021.
70. Si A, Misra AK: Concise synthesis of the pyruvic acid acetal containing pentasaccharide repeating unit of the cell wall O-antigen of *Escherichia coli* O156. *RSC Adv.* 2017, 7:49903–49909.
71. Gucchait A, Shit P, Misra AK: Synthesis of the tetrasaccharide repeating unit of the O-specific polysaccharide of *Azospirillum dobereineriae* type strain GSF71^T using linear and one-pot iterative glycosylations. *Beilstein J Org Chem* 2020, 16:1700–1705. [PubMed: 32733613]
72. Trattning N, Farcet JB, Gritsch P, Christler A, Pantophlet R, Kosma P: Synthesis of a pentasaccharide fragment related to the inner core region of rhizobial and agrobacterial lipopolysaccharides. *J Org Chem* 2017, 82:12346–12358. [PubMed: 29028168]
73. Chapman RN, Liu L, Boons GJ: 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. *J Am Chem Soc* 2018, 140:17079–17085. [PubMed: 30452253] **Pyruvylated oligosaccharides was synthesized and the 4,6-O-pyruvyl ketal group was confirmed to be crucial for glycan-protein binding.
74. Zhang X, Wang D, Jin G, Wang L, Guo Z, Gu G: Synthesis of a tetrasaccharide repeating unit of the exopolysaccharide from *Burkholderia multivorans*. *J Carbohydr Chem* 2017, 36:189–204.
75. Liu H, Zhang Y, Wei R, Andolina G, Li X: Total synthesis of *Pseudomonas aeruginosa* 1244 pilin glycan via de novo synthesis of pseudaminic acid. *J Am Chem Soc* 2017, 139:13420–13428. [PubMed: 28837324]
76. Lei J-C, Ruan Y-X, Luo S, Yang J-S: Stereodirecting effect of C3-ester groups on the glycosylation stereochemistry of L-rhamnopyranose thioglycoside donors: stereoselective synthesis of α - and β -L-rhamnopyranosides. *Eur J Org Chem* 2019, 2019:6377–6382. **A remote 4-nitro-picoloyl group in a L-rhamnosyl thioglycoside donor was found to improve the hydrogen-bond-mediated aglycon delivery (HAD) effect of picoloyl group for stereoselective formation of the challenging L-Rha- β 1–4Glc linkage.
77. Nishi N, Sueoka K, Iijima K, Sawa R, Takahashi D, Toshima K: Stereospecific beta-L-rhamnopyranosylation through an S_Ni -type mechanism by using organoboron reagents. *Angew Chem Int Ed Engl* 2018, 57:13858–13862. [PubMed: 30098095] **A 1,2-anhydro-L-rhamnopyranose donor and a boronic ester formed from a Glc diol acceptor and 4-nitrophenylboronic acid were used to form the challenging L-Rha- β 1–4Glc linkage in 88% yield with a complete stereospecific control and high regio-selectivity.
78. Behera A, Kulkarni SS: Chemical synthesis of rare, deoxy-amino sugars containing bacterial glycoconjugates as potential vaccine candidates. *Molecules* 2018, 23:1997.
79. Dimakos V, Taylor MS: Site-selective functionalization of hydroxyl groups in carbohydrate derivatives. *Chem Rev* 2018, 118:11457–11517.
80. Ghosh B, Kulkarni SS: Advances in protecting groups for oligosaccharide synthesis. *Chem Asian J* 2020, 15:450–462. [PubMed: 31895493]

81. Kulkarni SS, Wang CC, Sabbavarapu NM, Podilapu AR, Liao PH, Hung SC: "One-pot" protection, glycosylation, and protection-glycosylation strategies of carbohydrates. *Chem Rev* 2018, 118:8025–8104. [PubMed: 29870239]
82. Cheng CW, Zhou Y, Pan WH, Dey S, Wu CY, Hsu WL, Wong CH: Hierarchical and programmable one-pot synthesis of oligosaccharides. *Nat Commun* 2018, 9:5202. [PubMed: 30523255]
83. Rai D, Kulkarni SS: Recent advances in beta-L-rhamnosylation. *Org Biomol Chem* 2020, 18:3216–3228. [PubMed: 32270840]
84. Mi X, Lou Q, Fan W, Zhuang L, Yang Y: Gold(I)-catalyzed synthesis of beta-Kdo glycosides using Kdo ortho-hexynylbenzoate as donor. *Carbohydr Res* 2017, 448:161–165. [PubMed: 28478910]
85. Huang W, Zhou YY, Pan XL, Zhou XY, Lei JC, Liu DM, Chu Y, Yang JS: Stereodirecting effect of C5-carboxylate substituents on the glycosylation stereochemistry of 3-deoxy-D-manno-oct-2-ulonic acid (Kdo) thioglycoside donors: Stereoselective synthesis of alpha- and beta-Kdo glycosides. *J Am Chem Soc* 2018, 140:3574–3582. [PubMed: 29481074]
86. Ngoje P, Crich D: Stereocontrolled synthesis of the equatorial glycosides of 3-deoxy-D-manno-oct-2-ulonic acid: Role of side chain conformation. *J Am Chem Soc* 2020, 142:7760–7764. [PubMed: 32275429]
87. Dhakal B, Crich D: Synthesis and stereocontrolled equatorially selective glycosylation reactions of a pseudaminic acid donor: Importance of the side-chain conformation and regioselective reduction of azide protecting groups. *J Am Chem Soc* 2018, 140:15008–15015. [PubMed: 30351022] **A thioadamantyl (SAdA) Pse donor was rationally designed, predicted by conformational analysis, and confirmed experimentally to show an excellent stereoselectivity in synthesizing β -linked Pse-glycosides.
88. Panza M, Pistorio SG, Stine KJ, Demchenko AV: Automated chemical oligosaccharide synthesis: Novel approach to traditional challenges. *Chem Rev* 2018, 118:8105–8150. [PubMed: 29953217]
89. Emmadi M, Kulkarni SS: Recent advances in synthesis of bacterial rare sugar building blocks and their applications. *Nat Prod Rep* 2014, 31:870–879. [PubMed: 24700208]
90. Stowell SR, Arthur CM, McBride R, Berger O, Razi N, Heimbürg-Molinaro J, Rodrigues LC, Gourdine JP, Noll AJ, von Gunten S, et al.: Microbial glycan microarrays define key features of host-microbial interactions. *Nat Chem Biol* 2014, 10:470–476. [PubMed: 24814672]
91. Purohit S, Li T, Guan W, Song X, Song J, Tian Y, Li L, Sharma A, Dun B, Mysona D, et al.: Multiplex glycan bead array for high throughput and high content analyses of glycan binding proteins. *Nat Commun* 2018, 9:258–269. [PubMed: 29343722]
92. Garg M, Stern D, Gross U, Seeberger PH, Seeber F, Varon Silva D: Detection of anti-Toxoplasma gondii antibodies in human sera using synthetic glycosylphosphatidylinositol glycans on a bead-based multiplex assay. *Anal Chem* 2019, 91:11215–11222. [PubMed: 31401830]

Fig 1.
Monosaccharide building blocks of recently synthesized bacterial surface glycans.

A) Synthetic CPS Oligosaccharides

B) Synthetic LPS or LOS Oligosaccharides

C) Synthetic Oligosaccharides of Other Types of Bacterial Surface Glycans

Fig 2.

Structures of recently chemically synthesized oligosaccharides of bacterial **A**) CPSs (1–25); **B**) LPSs or LOSs (26–49); and **C**) other surface glycans including cell wall polysaccharides (50–53), EPS (54), lipoteichoic acid (55), pilin glycan (56), biofilm glycan (57), mycobacterial surface arabinomannan, and arabinogalactan (58–60). Bioassays that have been performed for individual synthetic targets are shown below the corresponding structures.

A) Stereoselective glycosylation for forming β -L-Rha in compounds 3, 30, and 53 B) Stereoselective glycosylation for forming α -L-FucNAc in compounds 9 and 15, and β -L-FucNAc in compound 26

C) Stereoselective glycosylation for forming β -ManNAcA in compound 15 D) Preparation of heptoses in compounds 17, 18, and 47 and stereoselective formation of 1,2-*cis* linkages

E) Preparation of Kdo and stereoselective formation of α -Kdo in compound 47 and β -Kdo in compound 23

F) De novo synthesis of pseudaminic acid (Pse) and construction of building blocks for compounds 56 and 33

G) Stereoselective glycosylation for forming furanoside in compounds 52 and 58

Fig 3.

Examples of glycosylation strategies in the synthesis of the oligosaccharides and analogs of bacterial surface polysaccharides including the formation the following challenging glycosylation linkages: β -L-Rha (A), α/β -L-FucNAc (B), β -ManNAcA (C), heptose (D), α/β -Kdo (E), α/β -Pseudaminic acid (Pse) (F), and furanosides (G).