Total synthesis of zwitterionic PS A2 oligosaccharides from Bacteroides fragilis

Li, X.; Pan-Gao, Q.; Wan, Y.; Zeng, F.; Qi, J.; Tu, Y.; ... ; Zhang, Q.

CHEMISTRY

Total synthesis of zwitterionic PS A2 oligosaccharides from *Bacteroides fragilis*

Xinxin Li[1], Qingzhi Pan-Gao[1], Yongyong Wan[1], Fuxing Zeng[1], Jianying Qi[1], Yuanhong Tu[1], Liming Wang[1], Zhen Wang[1], Jeroen D. C. Codée[2], Qingju Zhang[1,3]*

Zwitterionic polysaccharides have unique structures and can elicit T cell–dependent immune responses. To unravel their molecular mode of action and develop potential alternatives for carrier proteins in carbohydrate-based vaccines, we here describe a total synthesis of zwitterionic PS A2 oligosaccharides from *Bacteroides fragilis*. The pentasaccharide and decasaccharide bearing one or two repeating units were efficiently synthesized via convergent glycosylation strategy. The ingenious protecting groups have enabled the stereoselectivity of glycosylation. A unique synthetic approach was developed to introduce the chiral 3-hydroxybutanoic acid side chain on mannoheptose, which proved very challenging to form in this skeleton.

INTRODUCTION

Bacterial surface poly/oligosaccharides have been shown to be effective antigens for the development of antibacterial vaccines (*1*–*7*). Normally, standalone polysaccharides are T cell–independent antigens and can only elicit low-affinity immunoglobulin M (IgM) responses. This limitation of polysaccharide vaccines can be overcome by conjugation of the saccharides to a carrier protein, such as mutant cross-reacting material from diphtheria toxin, diphtheria toxoid, or tetanus toxoid (*8*, *9*). This conjugation can recruit T cell help and induce a T cell–dependent immune response, inducing a long-lasting and boostable IgG antibody repsonse and immunological memory (*10*, *11*). Clinically, glycoconjugate vaccines have been widely used to prevent bacterial infections, such as *Streptococcus pneumoniae* (PCV7, PCV13, PCV15, and PCV20), *Haemophilus influenzae*, *Neisseria meningitidis* (serogroups A, C, and ACWY), and *Salmonella typhi* (*7*, *9*, *12*–*16*). A considerable challenge in the development of glycoconjugate vaccines is carrier-induced epitope suppression (CIES), which appears reduced immunogenicity when preexisting immunity is present against the same carrier protein it comprises. CIES has been well documented in clinical studies and identified as a substantial risk factor for limited vaccine efficacy. The development of potential alternatives for carrier proteins is a substantial research field (*17*–*19*).

Zwitterionic polysaccharides (ZPSs) are the only class of carbohydrates that can induce a T cell–dependent immune response, a mode of action normally restricted to proteins and peptides (*20*–*23*). Upon uptake of ZPS into antigen-presenting cells, they can be partially depolymerized to ZPS fragments that can be loaded onto major histocompatibility class II (MHC-II) receptors (HLA-DR). The antigen MHC-II is presented to the cell surface and can bind to $CD4^+$ T cells through their αβ T cell receptors, generating an adaptive immune response. The activated $CD4^+$ T cells can release cytokines that can activate cytotoxic T lymphocyte cells and B cells to produce high-affinity IgG antibodies and memory B cells that are needed for a long-lasting immune response (Fig. 1A) (*23*–*31*). Furthermore, ZPSs have been used as carrier in the development of anticancer vaccines, and ZPSs carrying tumor-associated carbohydrate antigens, such as a Tn-PS A1 conjugate, an sialyl-Tn (STn)-PS A1 conjugate, and a Thomsen-Friedenreich (TF)–PS B conjugate have been generated and these were shown to be capable of eliciting a strong immune response (*32*–*36*). Thus, ZPSs may present potential alternatives for carrier proteins in the development of next-generation glycoconjugate vaccines.

Owing to their unique immune activity and potential applications, many ZPS oligosaccharides have been synthesized over the years (Fig. 1B). In 2007, the first synthetic study toward the PSA1 tetrasaccharide repeating unit was reported by van der Marel and co-workers, but the final deprotection was not achieved because of the low reactivity of C4-OH of galactose fragment and could not obtain enough fully protected tetrasacchride (*37*). Four years later, Seeberger and co-workers reported the first synthesis of the PS A1 tetrasaccharide repeating unit after switching the glycosylation strategy (*38*); subsequently, the groups of Andreana and Kulkarni also disclosed the synthesis of PS A1 tetrasaccharides in 2018 and 2021 (*39*, *40*); Codée and co-workers demonstrated the first total synthesis of a PS A1 dodecasaccharide, representing three repeating units in 2023 (*41*). Bundle and co-workers were the first to complete the assembly of Sp1 oligosaccharides, comprising one or two repeating units in 2010 (*42*, *43*). One year later, Codée and co-workers accomplished the assembly of the three possible frame-shifted trisaccharide Sp1-repeating units (*44*). Seeberger and co-workers reported the synthesis of conjugation-ready Sp1 trisaccharide in 2014 (*45*), and the corresponding conjugate vaccine candidates were obtained in 2018 (*46*). Kulkarni and co-workers described the synthesis of an Sp1 trisaccharide using a late-stage oxidation in 2021 (*47*). In 2019, Codée and co-workers reported on a set of synthetic Sp1 fragments, ranging from the trisaccharide to the dodecasaccharide. The three-dimensional (3D) structure and antibody binding capacity were also reported in (*48*). In 2022, Codée and co-workers reported the first total synthesis of Sp1 oligosaccharides, carrying the labile *O*-acetyl esters (*49*). Synthetic reports on other ZPS oligosaccharides of other bacteria, such as *Bacteroides fragilis* PS B (*50*), the lipoteichoic acid of *S. pneumoniae* (PnC) (*51*), *Morganella morganii* (*52*), *Shigella sonnei* (*53*), and *Photorhabdus temperata* ZPS (*54*, *55*) have also been disclosed. Despite the substantial advancements in ZPSs synthesis, there is no synthetic report for the synthesis of PS

[1]National Research Centre for Carbohydrate Synthesis, Jiangxi Normal University, 99 Ziyang Avenue, Nan Chang 330022, China. [2]Leiden Institute of Chemistry, Leiden University, Einsteinweg 55, 2333 CC Leiden, Netherlands. [3]Jiangxi Provincial Key Laboratory of Natural and Biomimetic Drugs Research, Jiangxi Normal University, Nan Chang 330022, China.
*Corresponding author. Email: zhangq@jxnu.edu.cn

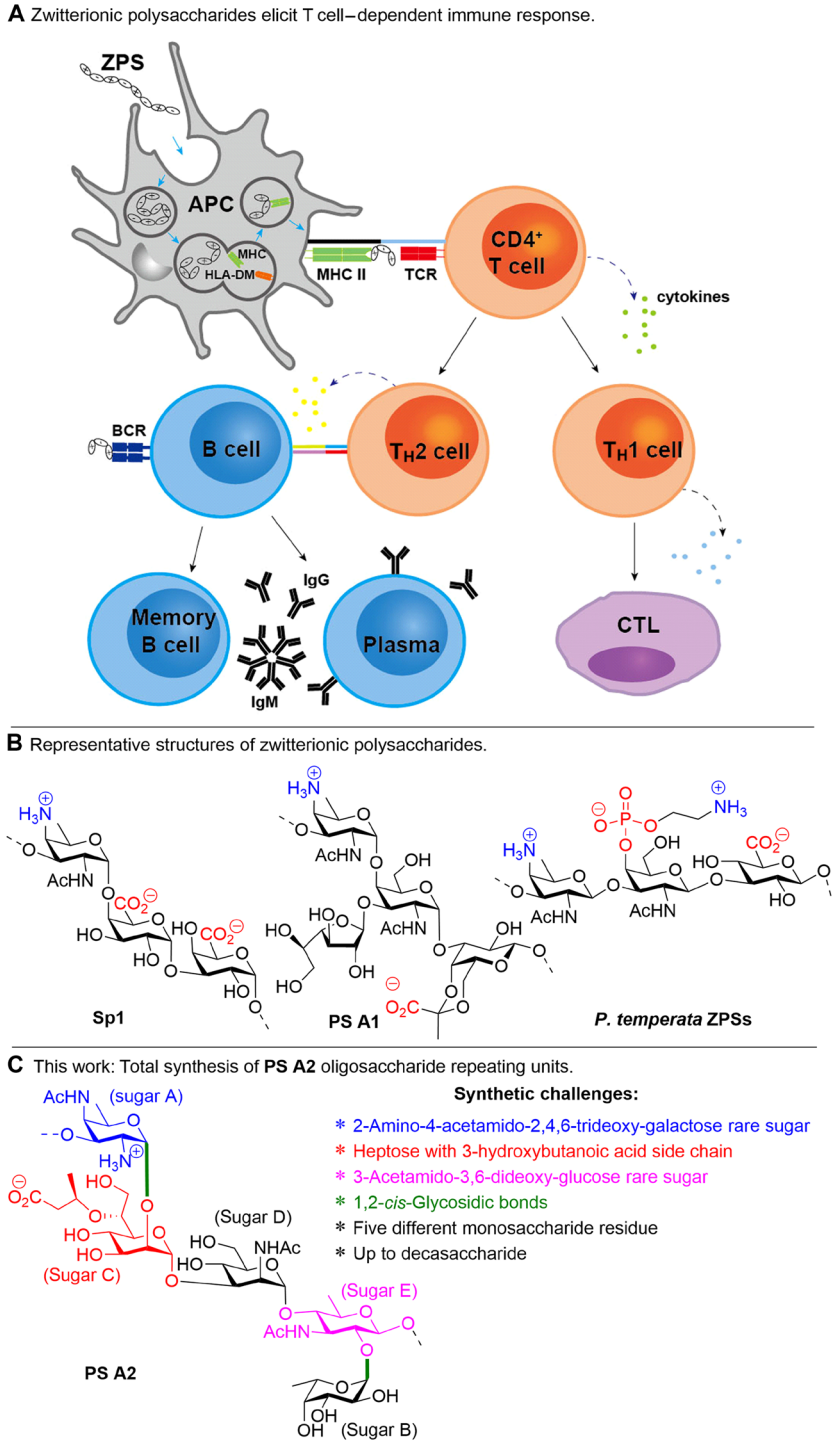

Fig. 1. Immunological activity and representative structures of ZPS. (**A**) ZPS elicit T cell–dependent immune response. (**B**) Representative structures of ZPS. (**C**) This work: Total synthesis of PS A2 oligosaccharide repeating units.

A2 owing to its complicated structure and synthetic challenges. Herein, we describe a total synthesis of PS A2 oligosaccharides encompassing one or two repeating units. The resulting oligosaccharides with appropriate amino spacers at the reducing ends are ready for microarray or surface plasmon resonance to enable biophysical interaction studies with MHC-II (Fig. 1C) (*56*, *57*).

RESULTS

The repeating unit of PS A2 is a branched pentasaccharide with a [→3)-α-D-FucN4NAc(1 → 2)-α-D-glycero-D-mannoHep(1 → 3)-α-D-ManNAc(1 → 4)-β-D-Qui3NAc(1→] backbone, carrying an α-L- fucose at the C2-OH of the Qui3NAc residue. The C6-OH of mannoheptose linked with a chiral 3-hydroxybutanoic acid through

an ether linkage (*58*). The unique structure of PS A2 presents many synthetic challenges: First, the repeating unit features the two rare sugars FucN4NAc and Qui3NAc, which are difficult to obtain (*59*). Second, the higher carbon sugar mannoheptose is substituted with a 3-hydroxy butanoic acid via an ether linkage (*60*–*63*). Third, the pentasaccharide repeating unit presents two demanding 1,2-*cis*-glycosidic linkages (*64*–*71*). Furthermore, the structure is highly functionalized, and it includes free amine, carboxylate and acetamido groups. To achieve an efficient synthesis, a convergent glycosylation strategy and orthogonal protecting groups were designed (Fig. 2). The targeted PS A2 fragment **1**, comprising two repeating units, could be obtained from the fully protected decasaccharide **3** via desilylation and hydrogenation steps. We planned to synthesize decasaccharide **3** through a [8 + (2 × 1)] glycosylation of octasaccharide diol **4** and L-fucose monosaccharide donor **5**, using the intrinsic nature of fucose to control the 1,2-*cis*-glycosidic bonds. The fully protected octasaccharide **6** could be constructed through a convergent [4 + 4] glycosylation using tetrasaccharide acceptor **7** and donor **9**.

In addition, the target pentasaccharide **2** can be obtained from tetrasaccharide **8** via delevulinoylation, [4 + 1] glycosylation and deprotection. The glycosylation of mannoheptosyl donor has shown its capability of good α selectivity without using any of neighboring group participation (*61*, *62*). Therefore, we planned to generate the tetrasaccharide building blocks **8** and **9** using disaccharide donor **10**, in combination with disaccharide acceptors **11** and **12**. The required disaccharides **10** to **12** can be generated from monosaccharide building blocks **13** to **17**. The acyl group on C4 position and bulky triisopropylsilyl (TIPS) group on C3 position of donor **13** are used to control 1,2-*cis* selectivity of glycosylation, while groups capable of neighboring group participation are placed on the C2 position of the donors to achieve the required 1,2-*trans* selectivity (*72*, *73*).

Our synthesis began with the preparation of monosaccharide building blocks **13** and **15** to **17** (Fig. 3). The rare sugar FucN4NAc building block **13** was assembled using a slight modification of Kulkarni's method, starting from 3-*O*-triisopropylsilyl protected β-D-rhamnose building block **18** (Fig. 3A) (*41*, *48*, *59*). Triflation of both the C2 and C4 hydroxy groups in **18**, subsequent inversion of the C2-triflate with tetrabutylammonium azide ($TBAN_3$), and substitution of the less reactive C4-OTf with ammonia, delivered the C4-NH_2 that was protected with a trichloroacetyl group (TCA), to give the core FucNNAc sugar structure **19** in 44% yield over four steps. The thioglycoside **19** was then smoothly transformed into the corresponding *N*-phenyl trifluoroacetimidate donor **13** in 62% yield over two steps (*74*). For the synthesis of the D-mannosamine donor, we started from the known D-glucose 2,3-diol **20** (Fig. 3B) (*75*). Regioselective triflation of C2-OH was followed by azide substitution using $TMSN_3$/KF to afford mannosazide **21** in 74% yield. The remaining hydroxyl group was masked as a 2-naphthylmethyl (NAP) ether, and subsequent removal of the benzylidene acetal and benzylation gave compound **22** in 94% yield. Reduction of the azides and protection of the resulting free amine were protected with a trichloroacetyl (TCA) group that generated compound **23**. The anomeric methyl group in **23** could be transformed into an acetyl group by use of Ac_2O and H_2SO_4 (*75*). Under the strongly acidic conditions, the C6-benzyl ether was concommitantly transformed into an acetyl ester. Last, the anomeric acetyl in **24** was selectively removed by hydrazine hydrate, and the liberated lactol was reacted with the imidoyl chloride to furnish monosaccharide donor **15′** in 68% yield over two steps.

The rare sugar Qui3NAc building block **16** bearing an anomeric linker was synthesized from commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose **25** (Fig. 3C). The C3 azido group was introduced smoothly using a previous method. Removal of the isopropylidene groups and acetylation then gave compound **26** in 48% over four steps. Introduction of a temporary 4-methoxyl phenyl group (MP) on the anomeric position afforded 3-azido-α-D-glucopyranose and followed by deacetylation gave **27** in 64%. Next, protection of the C4,6-diol with a benzylidene acetal, installation of the C-2 levulinoyl ester, and removal of the benzylidene group gave building block **28** in 68% over three steps. Regioselective tosylation of the primary alcohol furnished **29** in 76% yield. The remaining secondary hydroxyl group was masked as a *tert*-butyldimethylsilyl (TBS) ether using TBSOTf and 2,6-lutidine to obtain compound **30** in 74% yield. The tosyl group was then replaced for an iodide, after which the iodide and azide were reduced using tri-*n*-butyltin hydride/azodiisobutyronitrile at 85°C (*76*). Protection of the so generated free amine with trichloroacetyl chloride delivered **31** in 45% yield over three steps. Oxidative cleavage of the anomeric MP group liberated the hemiacetal, which could be transformed into *N*-phenyl trifluoroacetimidate donor **32** in 64% yield. The glycosylation of donor **32** and the 5-azidopentanol linker catalyzed by TBSOTf and subsequent removal of the TBS group with HF/pyridine afforded the desired Qui3NAc building block **16** in 66% yield. In addition, monosaccharide acceptor **17** was obtained from **31** in 86% yield via standard desilylation chemistry.

Next, we set out to synthesize the mannoheptose building block **14** as shown in Fig. 4. The properly protected mannoheptose **37** was obtained from D-mannose in 11 steps, and the newly formed chiral central at C6 was confirmed by X-ray diffraction (Fig. 4A) (*61*). The construction of the ether linkage between 3-hydroxy butanoic acid and the C6 alcohol of the mannoheptose proved to be challenging, and several approaches were tried. First, the canonical Williamson ether formation was investigated (Fig. 4B). Many reaction conditions for the connection of mannoheptose **37** and electrophilic reactants **38a-d** were explored, but the desired product could not be obtained. Next, the base-mediated Oxa-Michael addition was probed, which gave the desired product **42** as an R/S mixture in poor yield (Fig. 4C) (*77*, *78*). We then turned to a Pd(II)-catalyzed C(sp3)-H activation alkoxylation, which has been shown to afford ether linkages through a Pd(IV) intermediate (*79*, *80*). Compounds **43a-b** were used in combination with different bidentate ligands, but the desired products could hardly be detected. We also tried to use a two-step method, involving coupling of **43c-d** in the first Pd(II)-catalyzed C(sp3)-H activation alkoxylation step, and subsequent methylation, but this approach failed as well (*81*, *82*). Therefore, an indirect synthetic method was investigated using a three-step reaction sequence involving esterification, enol ether formation, and hydrogenation (Fig. 4E) (*83*, *84*). The esterification of the C6-OH in **37** proceeded smoothly to give **46** in 94% yield (*85*), and the so-formed ester was successfully converted into the enol ether **47** using dicyclopentadienyldimethyl titanium (Petasis reagent) in 85% yield (*84*). Hydrogenation using $Pd(OH)_2$/C with triethyl amine as additive then gave the desired ether as an inseparable mixture of diastereomers. Removal of the primary silyl group afforded the diastereomeric primary alcohols, which proved to be separable (**48R**:**48S** = 3:1).

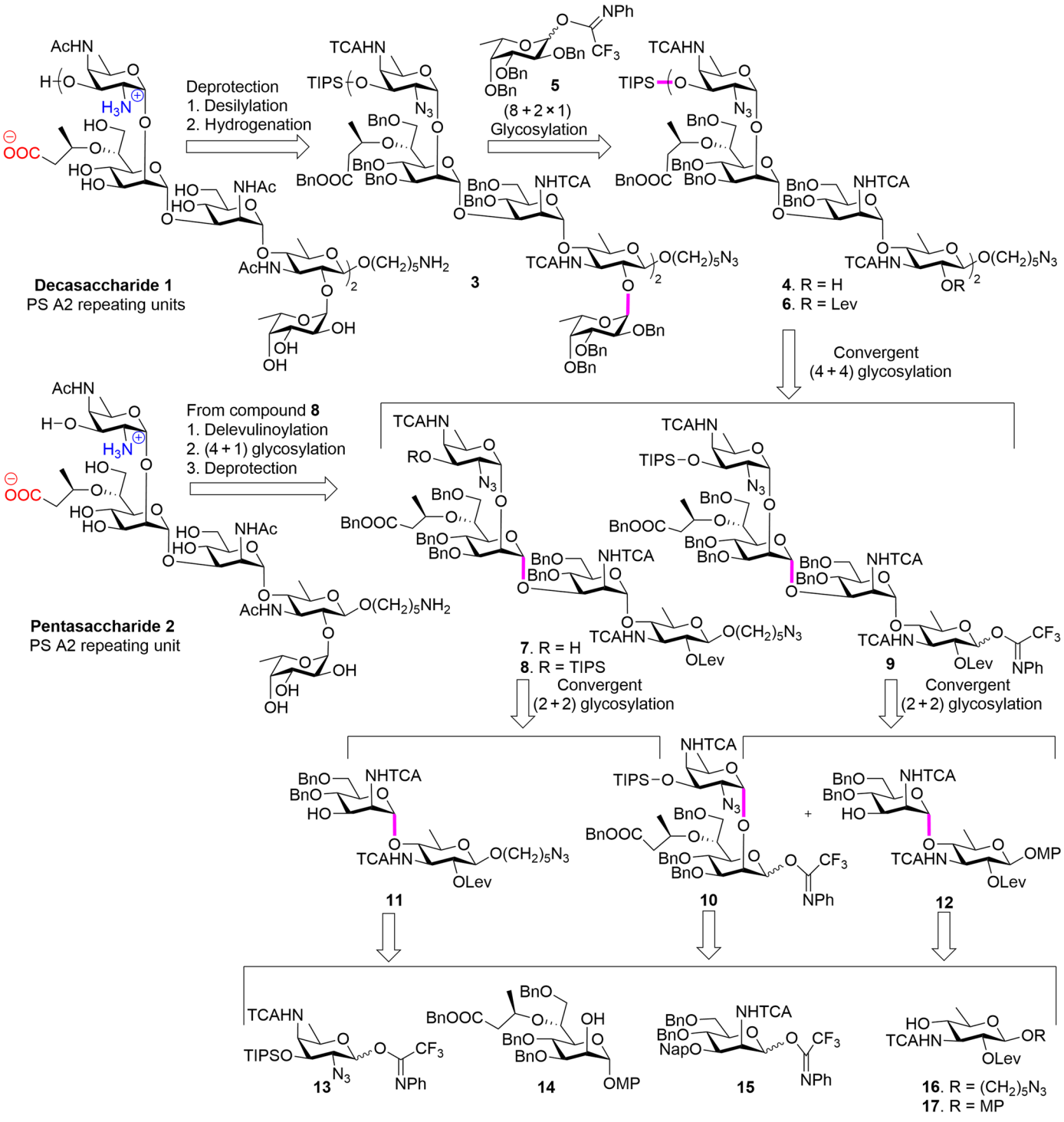

Fig. 2. The retrosynthesis analysis for PS A2 oligosaccharides 1 and 2.

Oxidation of the primary hydroxyl group in **48R** to the corresponding carboxylic acid using 2,2,6,6-tetramethyl-1-piperidinyloxyl/[bis(acetoxy)iodo]benzene (TEMPO/BAIB), and subsequent treatment with BnBr and $KHCO_3$ afforded benzyl ester **42R** in 81% yield. Removal of the isopropylidene group using 80% AcOH and following tin-mediated regioselective benzylation of the C3-OH using Bu_2SnO and BnBr afforded the desired mannoheptose building block **14** in 73% yield over three steps. The newly generated chiral center in the 3-hydroxybutanoic acid residue in compound **48** was confirmed by the formation of products **51a** and **51b**, which showed characteristic nuclear Overhauser effect (NOE) signals in nuclear magnetic resonance (NMR) (Fig. 4F).

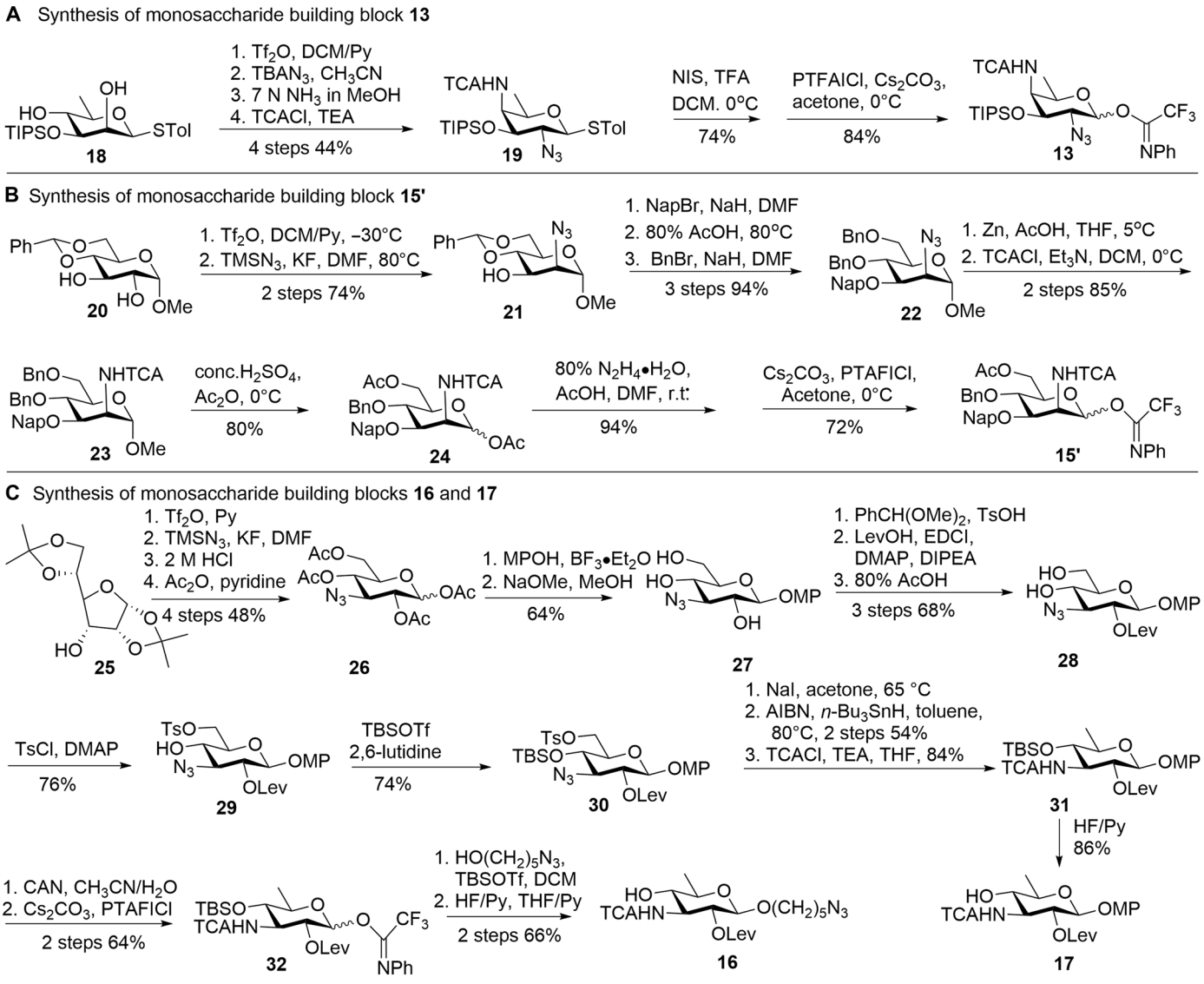

Fig. 3. Synthesis of monosaccharide building blocks 13, 15′, 16, and 17. (**A**) Synthesis of **13**; (**B**) Synthesis of **15′**; (**C**) Synthesis of **16**–**17**.

With all the monosaccharide building blocks in hand, the assembly of PS A2 pentasaccharide repeating unit **2** was undertaken using a convergent [2 + 2 + 1] glycosylation synthetic strategy as shown in Fig. 5. The glycosylation between the diaminofucose donor **13** and mannoheptose acceptor **14** using TBS triflate (TBSOTf) as a promotor furnished disaccharide **52** as single anomer in 83% yield. The stereoselectivity was benefited from the acyl group on C4 position and the bulky TIPS group on C3 hydroxyl of the donor **13**. Oxidative cleavage of the anomeric MP group in **52** gave the hemiacetal that could be transformed into the corresponding *N*-phenyl trifluoroacetimidate donor **10** in 66% yield. In parallel, mannosamine donor **15′** was glycosylated with 3-aminoquinovose acceptor **16** to generate the desired disaccharide **53** in 74% yield. Removal of the Nap group in **53** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave the disaccharide acceptor **11′** in 75% yield, which was coupled with disaccharide donor **10** to furnish tetrasaccharide **8′** in 65% yield with excellent stereoselectivity. Selectively removal of the levulinoyl group (Lev) in **8′** using hydrazine hydrate delivered the tetrasaccharide acceptor **54** in 82% yield. Last, the [4 + 1] glycosylation of this tetrasaccharide and fucose donor **5** using TBSOTf as a promotor gave fully protected pentasaccharide **55** in 84% yield as single anomer. Deprotection of the pentasaccharide was achieved by the removal of the silyl group by HF/pyridine, after which the $Pd(OH)_2$/C catalyzed hydrogenolysis of all benzyl ethers, the benzyl ester, and the concomitant transformation of the TCA into the corresponding acetamide and final hydrolysis of the acetate ester, to afford the target PS A2 pentasaccharide repeating unit **2** in 70% yield, over three steps.

Encouraged by the successful synthesis of the PS A2 pentasaccharide, we undertook the synthesis of the more challenging decasaccharide **1** containing two repeating units (Fig. 6). We started with the synthesis of tetrasaccharide donor **9′** (Fig. 6A). The glycosylation between mannosamine donor **15′** and 3-aminoquinovose acceptor **17** in the presence of TBSOTf furnished disaccharide **56** as

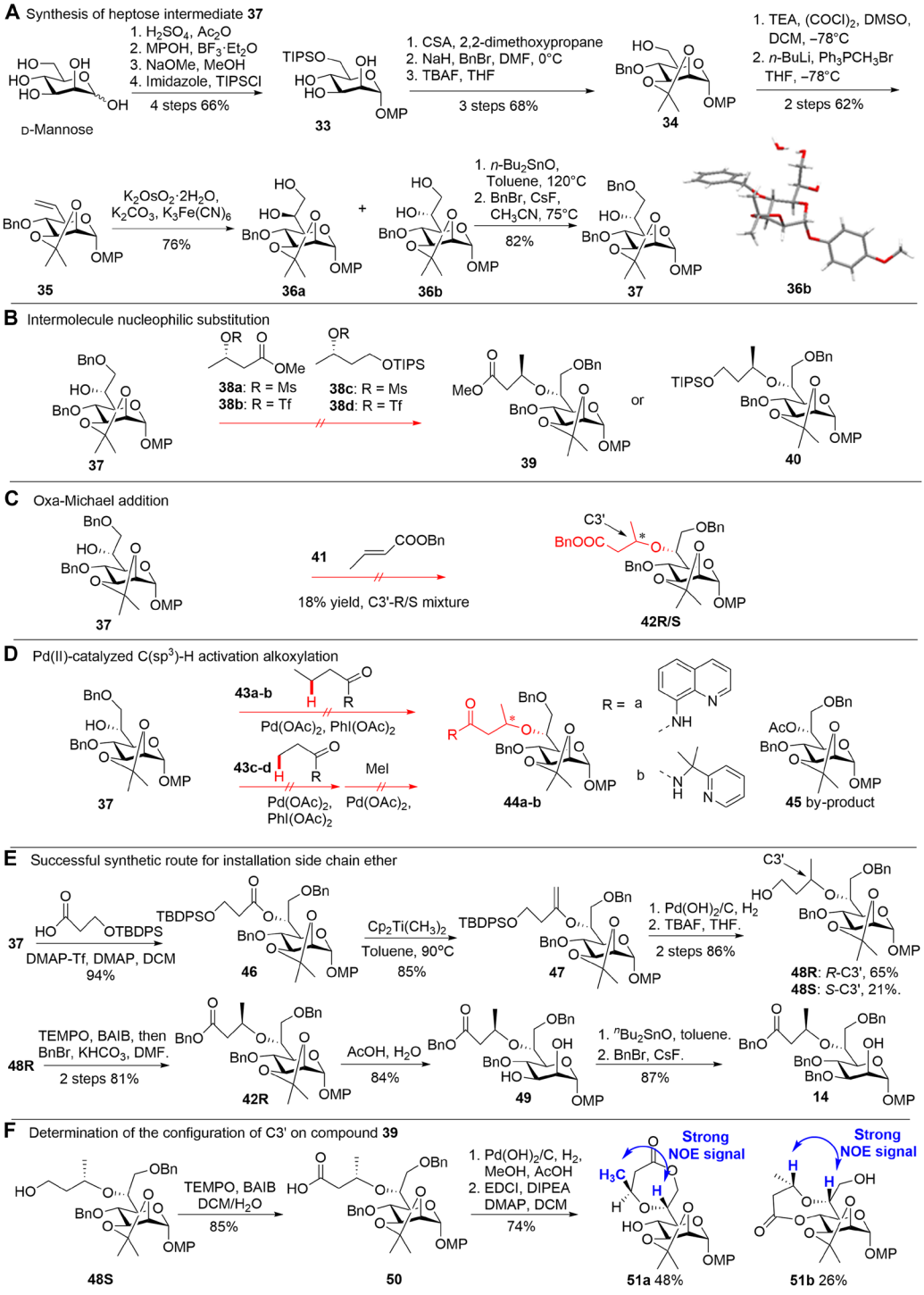

Fig. 4. Synthesis of mannoheptose building block 14. (**A**) Synthesis of heptose intermediate **37**; (**B**) Intermolecule nucleophilic substitution; (**C**) Oxa-Michael addition; (**D**) Pd(II)-catalyzed C(sp3)-H activation alkoxylation; (**E**) Successful synthetic route for installation side chain ether; (**F**) Determination of the configuration of C3′ on compound **39**.

single anomer in 90% yield. Oxidative cleavage of the Nap group using DDQ gave disaccharide acceptor **12′** in 78% yield, which was coupled with disaccharide donor **10** catalyzed by TBSOTf to furnish tetrasaccharide **57** as single anomer in 69% yield. Oxidative cleavage of the anomeric MP group in **57** gave the hemiacetal that could be transformed into the corresponding *N*-phenyl trifluoroacetimidate donor **9′** in 74% yield. The tetrasaccharide donor **9′** was glycosylated with tetrasaccharide acceptor **7′** obtained from tetrasaccharide **8′** to generate the desired octasaccharide **6′** in 65% yield. The octasaccharide diol acceptor **4′** was obtained by selective removal of the Lev groups in **6′** using hydrazine hydrate in 89% yield. Double fucosylation of **4′** adopting a [8 + (2 × 1)] glycosylation gave fully protected

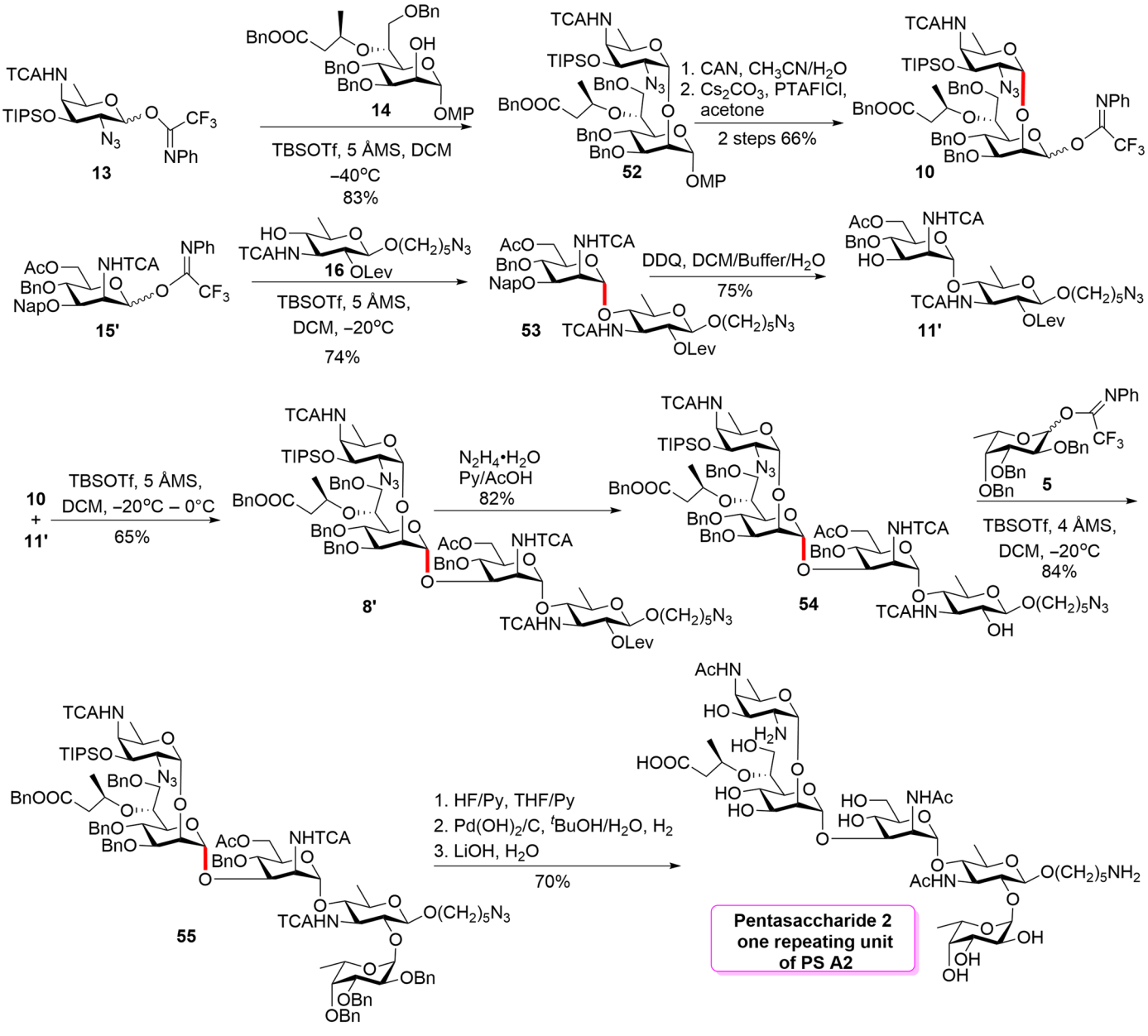

Fig. 5. Synthesis of target PS A2 repeating unit pentasaccharide 2.

decasaccharide **3′** in 67% yield with pure selectivity. Last, deprotection through desilylation, $Pd(OH)_2/C$ catalyzed hydrogenation, and subsequent basic acetate hydrolysis, afforded the target PS A2 decasaccharide **1** encompassing two repeating units in 62% yield, over three steps.

The structure of synthetic pentasaccharide **2** and decasaccharide **1** were next investigated using NMR data analysis. The $^{1}J_{C1,H1}$ coupling constants of synthetic oligosaccharides **1** and **2** are consistent with those established for native PS A2: pentasaccharide **2** sugar A (172 Hz), sugar B (170 Hz), sugar C (174 Hz), sugar D (172 Hz), and sugar E (162 Hz); decasaccharide **1** sugar A/A′ (173 Hz), sugar B/B′ (170 Hz), sugar C/C′ (172 Hz), sugar D/D′ (173 Hz), and sugar E/E′ (162 Hz). The NMR data of decasaccharide **1** are closer to the reported data for the native PS A2 than that of pentasaccharide **2** (fig. S1). For example, the ^{13}C chemical shifts difference ($^{13}C\Delta\delta$) for C1 of sugar E of pentasaccharide **2** is 2.1 parts per million (ppm), while the ^{13}C chemical shift difference for C1 of sugar E′1 of decasaccharide **1** is close to 0. However, both the ^{13}C chemical shift for C1 of sugar A (FucN4NAc) and the $^{1}H/^{13}C$ chemical shift for H1/C1 of sugar C (mannoHep) do not match well with the reported shifts for the native PS A2. The difference in chemical shift for the reducing end 3-aminoquinovose residue may be accounted for by the fact that the synthetic fragments carry a linker. The other chemical shift discrepancies may be explained by the influence of the oligosaccharide length and frame sequence. The synthetic pentasaccharide **2** and decasaccharide **1** with this frame sequence are not long enough

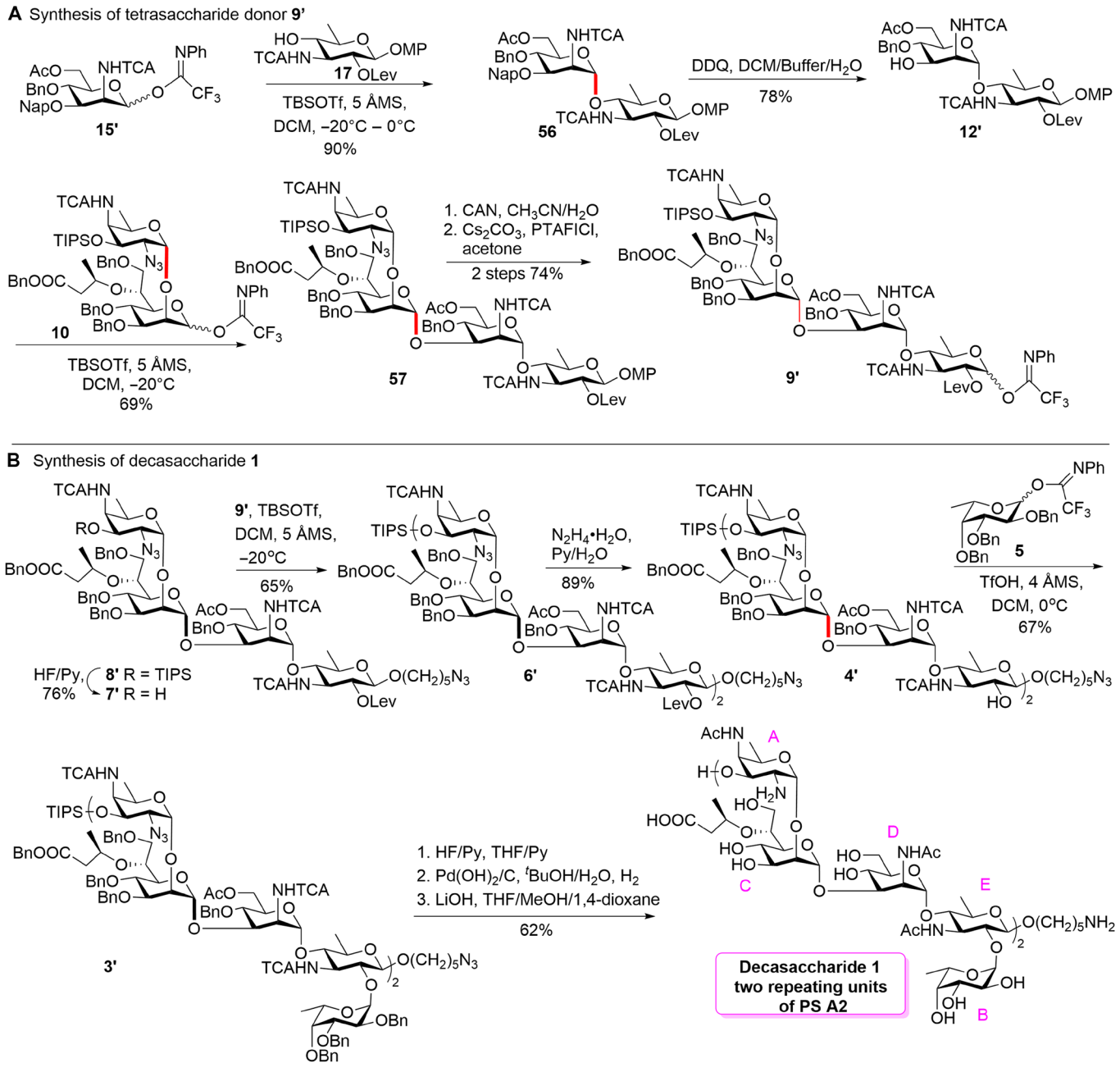

Fig. 6. Synthesis of target decasaccharide 1 containing two repeating units of PS A2. (**A**) Synthesis of tetrasaccharide donor 9′; (**B**) Synthesis of decasaccharide 1.

to form a stable 3D structure and take up a conformation that resembles the structure of the natural polysaccharide. The stereochemistry of the 3-hydroxybutanoic acid may also have influence. As we have also generated the 3-(*S*)-hydroxybutanoic acid functionalized mannoheptose building block **48S**, our synthetic approach will allow for the assembly of the oligomers with the alternative stereochemistry of the hydroxybutanoic acid appendage.

DISCUSSION

We have described a total synthesis of zwitterionic PS A2 oligosaccharides, comprising one and two repeating units. To enhance the synthetic efficiency of decasaccharide **1**, the octasaccharide backbone was assembled through a convergent [4 + 4] glycosylation, while the branching fucosyl residues were introduced in a double fucosylation event. The tetrasaccharide intermediates were obtained via a convergent [2 + 2] glycosylation. Stereoselectivity in the 1,2-*cis*-glycosylation reactions of the D-FucN4NAc donor was achieved by placing the acyl group on C4 position and a bulky TIPS group on the C3-hydroxyl of the donor. The formation of the ether linkage between the 3-hydroxybutanoic acid residue and the C-6 hydroxy of the mannoseheptose proved very challenging. Eventually, a three-step reaction sequence, involving ester formation, enol ether formation, and hydrogenation, proved effective to introduce the hydroxy butanoic acid side chain on the mannoheptose. The generated oligosaccharides carry amino spacers at their reducing end for the construction

of microarrays or attachment to surface plasmon resonance chips to enable biophysical interaction studies. The synthesis of longer PS A2 oligosaccharides and their analogs, their 3D structural analysis, and immunological activity studies will be reported in due course.

MATERIALS AND METHODS

All reagents were of commercial grade and used as received. All moisture sensitive reactions were performed under an argon/nitrogen atmosphere. The solvent (dichloromethane, toluene, ethyl ether, and CH_3CN) used in the glycosylation reactions was distilled over CaH_2 and stored on activated 4-Å molecular sieves before being used. Reactions were monitored by thin-layer chromatography analysis with detection by ultraviolet (254 nm) and where applicable by spraying with 20% sulfuric acid in EtOH or with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/liter) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/liter) in 10% sulfuric acid (aq.) followed by charring at ~150°C. Flash column chromatography was performed on silica gel (300 to 400 mesh). ^1H and ^{13}C spectra were recorded on a Bruker AV 400, Bruker AV 600 in $CDCl_3$, CD_3OD, CD_3COCD_3, or D_2O. Chemical shifts (δ) are given in parts per million relative to tetramethylsilane as internal standard (^1H NMR in $CDCl_3$) or the residual signal of the deuterated solvent. Coupling constants (*J*) are given in hertz. All ^{13}C spectra are proton decoupled. Where applicable, correlation spectroscopy, heteronuclear single-quantum coherence, NOE spectroscopy, heteronuclear multiple-bond correlation, and distortionless enhancement by polarization transfer (DEPT)135 experiments were used to further elucidate the structure. The anomeric product ratios were analyzed through integration of ^1H NMR signals or seperation. For the details of synthetic process, please see the Supplementary Materials.

Supplementary Materials

This PDF file includes:

Electronic Supplementary Information (ESI) available: details of experimental procedure and NMR spectra
Fig. S1
Tables S1 and S2

REFERENCES AND NOTES

1. A. Adibekian, P. Stallforth, M.-L. Hecht, D. B. Werz, P. Gagneux, P. H. Seeberger, Comparative bioinformatics analysis of the mammalian and bacterial glycomes. *Chem. Sci.* **2**, 337–344 (2011).
2. K. P. Klugman, I. T. Gilbertson, H. J. Koornhof, J. B. Robbins, R. Schneerson, D. Schulz, M. Cadoz, J. Armand, Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet* **2**, 1165–1169 (1987).
3. J. C. Parke, Capsular polysaccharide of *Haemophilus influenzae* type b as a vaccine. *Pediatr. Infect. Dis. J.* **6**, 795–798 (1987).
4. J. Eskola, A. Takala, H. Käyhty, *Haemophilus influenzae* type b polysaccharide-protein conjugate vaccines in children. *Curr. Opin. Pediatr.* **5**, 55–59 (1993).
5. M. Santosham, Prevention of *Haemophilus influenzae* type b disease. *Vaccine* **11**, S52–S57 (1993).
6. M. D. Decker, K. M. Edwards, *Haemophilus influenzae* type b vaccines: History, choice and comparisons. *Pediatr. Infect. Dis. J.* **17**, S113–S116 (1998).
7. P. H. Seeberger, Discovery of semi- and fully-synthetic carbohydrate vaccines against bacterial infections using a medicinal chemistry approach. *Chem. Rev.* **121**, 3598–3626 (2021).
8. M. G. Myers, The history of vaccine development and the diseases vaccines prevent in *Vaccinology: An Essential Guide*, G. N. M. a. A. D. T. Barrett., Eds., (John Wiley & Sons Ltd., 2015), pp. 1–31.
9. F. Micoli, L. Del Bino, R. Alfini, F. Carboni, M. R. Romano, R. Adamo, Glycoconjugate vaccines: Current approaches towards faster vaccine design. *Expert Rev. Vaccines* **18**, 881–895 (2019).
10. R. Adamo, Advancing homogeneous antimicrobial glycoconjugate vaccines. *Acc. Chem. Res.* **50**, 1270–1279 (2017).
11. R. Rappuoli, Glycoconjugate vaccines: Principles and mechanisms. *Sci. Transl. Med.* **10**, eaat4615 (2018).
12. R. Li, H. Yu, X. Chen, Recent progress in chemical synthesis of bacterial surface glycans. *Curr. Opin. Chem. Biol.* **58**, 121–136 (2020).
13. A. Saleh, S. Qamar, A. Tekin, R. Singh, R. Kashyap, Vaccine development throughout history. *Cureus* **13**, e16635 (2021).
14. Y. Hu, X. Zhang, S. Deng, C. Yue, X. Jia, Y. Lyu, Non-antibiotic prevention and treatment against *Acinetobacter baumannii* infection: Are vaccines and adjuvants effective strategies? *Front. Microbiol.* **14**, 1049917 (2023).
15. R. Rohokale, Z. Guo, Development in the concept of bacterial polysaccharide repeating unit-based antibacterial conjugate vaccines. *ACS Infect. Dis.* **9**, 178–212 (2023).
16. L. Del Bino, K. E. Østerlid, D.-Y. Wu, F. Nonne, M. R. Romano, J. Codée, R. Adamo, Synthetic glycans to improve current glycoconjugate vaccines and fight antimicrobial resistance. *Chem. Rev.* **122**, 15672–15716 (2022).
17. H. Zhu, C. S. Rollier, A. J. Pollard, Recent advances in lipopolysaccharide-based glycoconjugate vaccines. *Expert Rev. Vaccines* **20**, 1515–1538 (2021).
18. M. J. McCluskie, D. M. Evans, N. Zhang, M. Benoit, S. P. McElhiney, M. Unnithan, S. C. DeMarco, B. Clay, C. Huber, A. Deora, J. M. Thorn, D. R. Stead, J. R. Merson, H. L. Davis, The effect of preexisting anti-carrier immunity on subsequent responses to CRM197or Qb-VLP conjugate vaccines. *Immunopharmacol. Immunotoxicol.* **38**, 184–196 (2016).
19. M. Tashani, M. Alfelali, O. Barasheed, A. S. Alqahtani, L. Heron, M. Wong, H. Rashid, H. Findlow, R. Borrow, R. Booy, Effect of Tdap upon antibody response to meningococcal polysaccharide when administered before, with or after the quadrivalent meningococcal TT-conjugate vaccine (coadministered with the 13-valent pneumococcal CRM197-conjugate vaccine) in adult Hajj pilgrims: A randomised controlled trial. *Vaccine* **36**, 4375–4382 (2018).
20. A. Tzianabos, A. B. Onderdonk, B. Rosner, R. L. Cisneros, D. L. Kasper, Structural features of polysaccharides that induce intra-abdominal abscesses. *Science* **262**, 416–419 (1993).
21. A. Tzianabos, J. Y. Wang, D. L. Kasper, Biological chemistry of immunomodulation by zwitterionic polysaccharides. *Carbohydr. Res.* **338**, 2531–2538 (2003).
22. B. A. Cobb, D. L. Kasper, Characteristics of carbohydrate antigen binding to the presentation protein HLA-DR. *Glycobiology* **18**, 707–718 (2008).
23. N. K. Surana, D. L. Kasper, The yin yang of bacterial polysaccharides: Lessons learned from B. fragilis PSA. *Immunol. Rev.* **245**, 13–26 (2012).
24. B. A. Cobb, Q. Wang, A. O. Tzianabos, D. L. Kasper, Polysaccharide processing and presentation by the MHCII pathway. *Cell* **117**, 677–687 (2004).
25. S. K. Mazmanian, D. L. Kasper, The love-hate relationship between bacterial polysaccharides and the host immune system. *Nat. Rev. Immunol.* **6**, 849–858 (2006).
26. Q. Wang, R. M. McLoughlin, B. A. Cobb, M. Charrel-Dennis, K. J. Zaleski, D. Golenbock, A. O. Tzianabos, D. L. Kasper, A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. *J. Exp. Med.* **203**, 2853–2863 (2006).
27. L. S. Kreisman, J. H. Friedman, A. Neaga, B. A. Cobb, Structure and function relations with a T-cell-activating polysaccharide antigen using circular dichroism. *Glycobiology* **17**, 46–55 (2007).
28. T. L. Stephen, L. Groneck, W. M. Kalka-Moll, The modulation of adaptive immune responses by bacterial zwitterionic polysaccharides. *Int. J. Microbiol.* **2010**, 917075 (2010).
29. E. B. Troy, D. L. Kasper, Beneficial effects of Bacteroides fragilis polysaccharides on the immune system. *Front. Biosci.* **15**, 25–34 (2011).
30. L. Sun, D. R. Middleton, P. L. Wantuch, A. Ozdilek, F. Y. Avci, Carbohydrates as T-cell antigens with implications in health and disease. *Glycobiology* **26**, 1029–1040 (2016).
31. J. L. Johnson, M. B. Jones, B. A. Cobb, Polysaccharide A from the capsule of *Bacteroides fragilis* induces clonal $CD4^+$ T cell expansion. *J. Biol. Chem.* **290**, 5007–5014 (2015).
32. R. A. D. Silva, Q. Wang, T. Chidley, D. K. Appulage, P. R. Andreana, Immunological response from an entirely carbohydrate antigen: Design of synthetic vaccines based on Tn-PS A1 conjugates. *J. Am. Chem. Soc.* **131**, 9622–9623 (2009).
33. M. Shi, K. A. Kleski, K. R. Trabbic, J.-P. Bourgault, P. R. Andreana, Sialyl-Tn polysaccharide A1 as an entirely carbohydrate immunogen: Synthesis and immunological evaluation. *J. Am. Chem. Soc.* **138**, 14264–14272 (2016).
34. K. R. Trabbic, J.-P. Bourgault, M. Shi, M. Clark, P. R. Andreana, Immunological evaluation of the entirely carbohydrate-based Thomsen-Friedenreich – PS B conjugate. *Org. Bio. Chem.* **14**, 3350–3355 (2016).
35. S. Ghosh, K. R. Trabbic, M. Shi, S. Nishat, P. Eradi, K. A. Kleski, P. R. Andreana, Chemical synthesis and immunological evaluation of entirely carbohydrate conjugate Globo H-PS A1. *Chem. Sci.* **11**, 13052–13059 (2020).
36. K. A. Kleski, K. R. Trabbic, M. Shi, J.-P. Bourgault, P. R. Andreana, Enhanced immune response against the Thomsen-Friedenreich tumor antigen using a bivalent entirely carbohydrate conjugate. *Molecules* **25**, 1319 (2020).
37. L. J. van den Bos, T. J. Boltje, T. Provoost, J. Mazurek, H. S. Overkleeft, G. A. van der Marel, A synthetic study towards the PSA1 tetrasaccharide repeating unit. *Tetrahedron Lett.* **48**, 2697–2700 (2007).

38. R. Pragani, P. H. Seeberger, Total synthesis of the *Bacteroides fragilis* zwitterionic polysaccharide A1 repeating unit. *J. Am. Chem. Soc.* **133**, 102–107 (2011).
39. P. Eradi, S. Ghosh, P. R. Andreana, 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.* **20**, 4526–4530 (2018).
40. E. K. Pathan, B. Ghosh, A. R. Podilapu, S. S. Kulkarni, Total synthesis of the repeating unit of *Bacteroides fragilis* zwitterionic polysaccharide A1. *J. Org. Chem.* **86**, 6090–6099 (2021).
41. Z. Wang, A. Poveda, Q. Zhang, L. Unione, H. S. Overkleeft, G. A. van der Marel, J. B. Jesus, J. D. C. Codee, Total synthesis and structural studies of zwitterionic *Bacteroides fragilis* polysaccharide A1 fragments. *J. Am. Chem. Soc.* **145**, 14052–14063 (2023).
42. X. Wu, L. Cui, T. Lipinski, D. R. Bundle, Synthesis of monomeric and dimeric repeating units of the zwitterionic type 1 capsular polysaccharide from Streptococcus pneumoniae. *Chem. A Eur. J.* **16**, 3476–3488 (2010).
43. I. Iynkkaran, D. R. Bundle, Synthesis of a trisaccharide repeat of the zwitterionic Sp1 capsular polysaccharide utilizing 2-Azido-4-benzylamino-4N,3-O-carbonyl-2,4,6-trideoxy-d-galactopyranosyl trichloroacetimidate. *Carbohydr. Res.* **378**, 26–34 (2013).
44. A. E. Christina, L. J. van den Bos, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, Galacturonic acid lactones in the synthesis of all trisaccharide repeating units of the zwitterionic polysaccharide Sp1. *J. Org. Chem.* **76**, 1692–1706 (2011).
45. B. Schumann, R. Pragani, C. Anish, C. L. Pereira, P. H. Seeberger, Synthesis of conjugation-ready zwitterionic oligosaccharides by chemoselective thioglycoside activation. *Chem. Sci.* **5**, 1992–2002 (2014).
46. B. Schumann, K. Reppe, P. Kaplonek, A. Wahlbrink, C. Anish, M. Witzenrath, C. L. Pereira, P. H. Seeberger, Development of an efficacious, semisynthetic glycoconjugate vaccine candidate against *Streptococcus pneumoniae* serotype 1. *ACS Cent. Sci.* **4**, 357–361 (2018).
47. A. Ghosh, S. S. Kulkarni, Total synthesis of the repeating unit of *Streptococcus pneumoniae* zwitterionic polysaccharide Sp1. *J. Org. Chem.* **86**, 18292–18299 (2021).
48. Q. Zhang, A. Gimeno, D. Santana, Z. Wang, Y. Valdes-Balbin, L. M. Rodriguez-Noda, T. Hansen, L. Kong, M. Shen, H. S. Overkleeft, V. Verez-Bencomo, G. A. van der Marel, J. Jimenez-Barbero, F. Chiodo, J. D. C. Codee, Synthetic, zwitterionic Sp1 oligosaccharides adopt a helical structure crucial for antibody interaction. *ACS. Cent .Sci.* **5**, 1407–1416 (2019).
49. Z. Wang, A. Gimeno, M. G. Lete, H. S. Overkleeft, G. A. van der Marel, F. Chiodo, J. Jiménez-Barbero, J. D. C. Codée, Synthetic zwitterionic *Streptococcus pneumoniae* type 1 oligosaccharides carrying labile *O*-acetyl esters. *Angew. Chem. Int. Ed. Engl.* **62**, e202211940 (2022).
50. K. Puri, S. S. Kulkarni, Total synthesis of a structurally complex zwitterionic hexasaccharide repeating unit of polysaccharide B from *Bacteroides fragilis* via one-pot glycosylation. *Commun. Chem.* **7**, 204 (2024).
51. C. M. Pedersen, I. Figueroa-Perez, B. Lindner, A. J. Ulmer, U. Zahringer, R. R. Schmidt, Total synthesis of lipoteichoic acid of *Streptococcus pneumoniae*. *Angew. Chem. Int. Ed. Engl.* **49**, 2585–2590 (2010).
52. D. J. Keith, S. D. Townsend, Total synthesis of the congested, bisphosphorylated *Morganella morganii* zwitterionic trisaccharide repeating unit. *J. Am. Chem. Soc.* **141**, 12939–12945 (2019).
53. H. B. Pfister, L. A. Mulard, Synthesis of the zwitterionic repeating unit of the O-antigen from *Shigella sonnei* and chain elongation at both ends. *Org. Lett.* **16**, 4892–4895 (2014).
54. J. M. Nguyen, S. D. Townsend, Total synthesis of the *Photorhabdus temperata* ssp. Cinereal 3240 zwitterionic trisaccharide repeating unit. *Org. Lett.* **23**, 5922–5926 (2021).
55. K. Puri, S. S. Kulkarni, Total synthesis of the phosphorylated zwitterionic trisaccharide repeating unit of *Photorhabdus temperata* cinerea 3240. *Org. Lett.* **23**, 7083–7087 (2021).
56. Y. Qi, C. Qin, S. Zhu, J. Yin, Chemoselectivity of 3,3′-dithiobis(sulfosuccinimylpropionate)-based 3-mercaptopropionylation of amine-linked aminomonosaccharides. *J. Carbohydr. Chem.* **42**, 59–91 (2023).
57. M. Ding, C. Qin, W. Sun, G. Tian, X. Zou, J. Hu, J. Yin, Chemical synthesis of a densely functionalized aminodisaccharide from *Fusobacterium nucleatum* ATCC 23726 O-antigen. *Org. Lett.* **26**, 4142–4146 (2024).
58. Y. Wang, W. M. Kalka-Moll, M. H. Roehrl, D. L. Kasper, Structural basis of the abscess-modulating polysaccharide A2 from *Bacteroides fragilis*. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13478–13483 (2000).
59. M. Emmadi, S. S. Kulkarni, Recent advances in synthesis of bacterial rare sugar building blocks and their applications. *Nat. Prod. Rep.* **31**, 870–879 (2014).
60. C.-I. Lin, R. M. McCarty, H.-w. Liu, The biosynthesis of nitrogen-, sulfur-, and high-carbon chain-containing sugars. *Chem. Soc. Rev.* **42**, 4377–4407 (2013).
61. G. Tian, J. Hu, C. Qin, L. Li, X. Zou, J. Cai, P. H. Seeberger, J. Yin, Chemical synthesis and immunological evaluation of *Helicobacter pylori* serotype O6 tridecasaccharide O-antigen containing a dd-heptoglycan. *Angew. Chem. Int. Ed. Engl.* **59**, 13362–13370 (2020).
62. X. Zou, J. Hu, M. Zhao, C. Qin, Y. Zhu, G. Tian, J. Cai, P. H. Seeberger, J. Yin, Chemical synthesis of the highly sterically hindered core undecasaccharide of *Helicobacter pylori* lipopolysaccharide for antigenicity evaluation with human serum. *J. Am. Chem. Soc.* **144**, 14535–14547 (2022).
63. T. Li, J. Wang, X. Zhu, X. Zhou, S. Sun, P. Wang, H. Cao, G. Yu, M. Li, Synthesis of rare 6-deoxy-d-/l-heptopyranosyl fluorides: Assembly of a hexasaccharide corresponding to *Campylobacter jejuni* strain CG8486 capsular polysaccharide. *J. Am. Chem. Soc.* **143**, 11171–11179 (2021).
64. V. D. Alexei, 1,2-cis O-Glycosylation: Methods, strategies, principles. *Curr. Org. Chem.* **7**, 35–79 (2003).
65. L. Wang, H. S. Overkleeft, G. A. van der Marel, J. D. C. Codée, Reagent controlled stereoselective synthesis of α-glucans. *J. Am. Chem. Soc.* **140**, 4632–4638 (2018).
66. G. Báti, J.-X. He, K. B. Pal, X.-W. Liu, Stereo- and regioselective glycosylation with protection-less sugar derivatives: An alluring strategy to access glycans and natural products. *Chem. Soc. Rev.* **48**, 4006–4018 (2019).
67. J. Ling, C. S. Bennett, Recent developments in stereoselective chemical glycosylation. *Asian. J. Org. Chem.* **8**, 802–813 (2019).
68. R. A. Jeanneret, S. E. Johnson, M. C. Galan, Conformationally constrained glycosyl donors as tools to control glycosylation outcomes. *J. Org. Chem.* **85**, 15801–15826 (2020).
69. L. Duan, Q. Nie, Y. Hu, L. Wang, K. Guo, Z. Zhou, X. Song, Y. Tu, H. Liu, T. Hansen, J. S. Sun, Q. Zhang, Stereoselective synthesis of the O-antigen of *A. baumannii* ATCC 17961 using long-range levulinoyl group participation. *Angew. Chem. Int. Ed. Engl.* **62**, e202306971 (2023).
70. X. Wu, J. Lai, K. Yang, Y. Xue, J. Liao, L. Wang, J. Sun, J. Hu, J. Yin, Q. Zhang, Stereoselective synthesis and immunological evaluation of common O-antigen of *P. mirabilis* OE and *P. vulgaris* TG103. *Chin. J. Chem.* **41**, 535–543 (2023).
71. L. Zhang, Z. Zheng, Y. Zhang, X. Wu, Y. Tu, C. Liu, Z. Wang, L. Wang, Y. Yang, Q. Zhang, Chemical synthesis and antigenic evaluation of oligosaccharides of *Bordetella hinzii* O-antigen containing unique amidated 2,3-diacetamido-2,3-dideoxy-alduronic acids. *JACS Au* **5**, 1903–1913 (2025).
72. T. G. Frihed, M. Bols, C. M. Pedersen, Mechanisms of glycosylation reactions studied by low-temperature nuclear magnetic resonance. *Chem. Rev.* **115**, 4963–5013 (2015).
73. A. A. Hettikankanamalage, R. Lassfolk, F. S. Ekholm, R. Leino, D. Crich, Mechanisms of stereodirecting participation and ester migration from near and far in glycosylation and related reactions. *Chem. Rev.* **120**, 7104–7151 (2020).
74. B. Yu, J. Sun, Glycosylation with glycosyl N-phenyltrifluoroacetimidates (PTFAI) and a perspective of the future development of new glycosylation methods. *Chem. Commun.* **46**, 4668–4679 (2010).
75. C. Alex, S. Visansirikul, Y. Zhang, J. P. Yasomanee, J. Codee, A. V. Demchenko, Synthesis of 2-azido-2-deoxy- and 2-acetamido-2-deoxy-D-manno derivatives as versatile building blocks. *Carbohydr. Res.* **488**, 107900 (2020).
76. D. S. Hays, G. C. Fu, Development of Bu_3SnH-catalyzed processes: Efficient reduction of azides to amines. *J. Org. Chem.* **63**, 2796–2797 (1998).
77. Y. Wang, D.-M. Du, Recent advances in organocatalytic asymmetric oxa-Michael addition triggered cascade reactions. *Org. Chem. Front.* **7**, 3266–3283 (2020).
78. J. Hu, M. Bian, H. Ding, Recent application of oxa-Michael reaction in complex natural product synthesis. *Tetrahedron Lett.* **57**, 5519–5539 (2016).
79. F.-J. Chen, S. Zhao, F. Hu, K. Chen, Q. Zhang, S.-Q. Zhang, B.-F. Shi, Pd(ii)-catalyzed alkoxylation of unactivated C(sp3)–H and C(sp2)–H bonds using a removable directing group: Efficient synthesis of alkyl ethers. *Chem. Sci.* **4**, 4187–4192 (2013).
80. S.-Y. Zhang, G. He, Y. Zhao, K. Wright, W. A. Nack, G. Chen, Efficient alkyl ether synthesis via palladium-catalyzed, picolinamide-directed alkoxylation of unactivated C(sp3)–H and C(sp2)–H bonds at remote positions. *J. Am. Chem. Soc.* **134**, 7313–7316 (2012).
81. C. Chen, G. C. Fu, Copper-catalysed enantioconvergent alkylation of oxygen nucleophiles. *Nature* **618**, 301–307 (2023).
82. H. Liu, Q. Huang, R.-Z. Liao, M. Li, Y. Xie, Ring-closing C–O/C–O metathesis of ethers with primary aliphatic alcohols. *Nat. Commun.* **14**, 1883 (2023).
83. T. L. Diguarher, D. C. Billington, G. Dorey, A convenient synthesis of isopropyl ethers from secondary alcohols. *Syn. Commun.* **25**, 1633–1639 (1995).
84. R. C. Hartley, G. J. McKiernan, Titanium reagents for the alkylidenation of carboxylic acid and carbonic acid derivatives. *J. Chem. Soc., Perkin Trans.* **1**, 2763–2793 (2002).
85. D. Chen, L. Xu, B. Ren, Z. Wang, C. Liu, Triflylpyridinium as coupling reagent for rapid amide and ester synthesis. *Org. Lett.* **25**, 4571–4575 (2023).

Acknowledgments

Funding: This work was financially supported by the National Natural Science Foundation of China (22377043, 22167015, and 21977039) and the Science and Technology Department of Jiangxi Province (20244BDF60014, jxsq2020101084, 20224ACB213006, and 2024SSY07051). The recipient is Q.Z. **Author contributions:** Conceptualization: Q.Z. Methodology: X.L., Q.P.-G., Y.W., J.Q., and Y.T. Investigation: X.L., Q.P-G., Y.W., F.Z., and Q.Z. Supervision: L.W. and Q.Z. Writing—original draft: X.L. and Q.Z. Writing—review and editing: Z.W., J.D.C.C., and Q.Z. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 12 August 2025
Accepted 7 November 2025
Published 12 December 2025
10.1126/sciadv.aeb4065