Synthesis of orthogonally protected bacterial, rare-sugar and D-glycosamine building blocks

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Bacterial glycoconjugates comprise atypical deoxy amino sugars that are not present on the human cell surface, making them good targets for drug discovery and carbohydrate-based vaccine development. Unfortunately, they cannot be isolated with sufficient purity in acceptable amounts, and therefore chemical synthesis is a crucial step toward the development of these products. Here we describe a detailed protocol for the synthesis of orthogonally protected bacterial deoxy amino hexopyranoside (2,4-diacetamido-2,4,6-trideoxyhexose (DATDH), D-bacillosamine, D-fucosamine, and 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT)), D-glucosamine and D-galactosamine building blocks starting from β-D-thiophenylmannoside. Readily available β-D-thiophenylmannoside was first converted into the corresponding 2,4-diols via deoxygenation or silylation at C6, followed by O3 acylation. The 2,4-diols were converted into 2,4-bis-trifluoromethanesulfonates, which underwent highly regioselective, one-pot, double-serial and double-parallel displacements by azide, phthalimide, acetate and nitrite ions as nucleophiles. Thus, D-rhamnosyl- and D-mannosyl 2,4-diols can be efficiently transformed into various rare sugars and D-galactosamine, respectively, as orthogonally protected thioglycoside building blocks on a gram scale in 1–2 d, in 54–85% overall yields, after a single chromatographic purification. This would otherwise take 1–2 weeks. D-Glucosamine building blocks can be prepared from β-D-thiophenylmannoside in four steps via C2 displacement of triflates by azide in 2 d and in 66–70% overall yields. These procedures have been applied to the synthesis of L-serine-linked trisaccharide of *Neisseria meningitidis* and a rare disaccharide fragment of the zwitterionic polysaccharide (ZPS) A1 (ZPS A1) of *Bacteroides fragilis*.

INTRODUCTION

Glycosylation, the post-translational modification of proteins by carbohydrates to modulate protein structure and function, was once believed to be restricted to eukaryotes¹. Over the past 20 years, this notion has been refuted, and now it is well established that protein glycosylation is also common in prokaryotes². However, the two differ in their glycan structures. The bacterial glycoconjugates comprise atypical deoxy amino sugars, which are not present on the human cell surface. This peculiar structural difference allows discrimination between the pathogen and the host cell and offers avenues for target-specific drug discovery and carbohydrate-based vaccine development^{3–5}.

In contrast to their eukaryotic counterparts that contain N-acetyl D-galactosamine (GalNAc) at the reducing end, bacterial glycoproteins have unusual monosaccharides attached to L-serine or L-threonine (Fig. 1), whereas the branched GalNAc or N-acetyl D-glucosamine (GlcNAc) residues are placed at the interior sites and are connected to various other sugars at O3, O4 or O6 positions. For example, O-linked glycans located on the pilin of *N. gonorrhoeae* and *N. meningitidis* consist of DATDH (Fig. 1a; ref. 6), whereas N-linked glycans of *Campylobacter jejuni*⁷ comprise a bacillosamine core (Fig. 1b) attached to a branched GalNAc pentasaccharide. Similarly, O-linked glycans on flagellin of *Pseudomonas aeruginosa* contain N-acetyl fucosamine (FucNAc) (Fig. 1c; ref. 8). In addition, several ZPSs located on the surfaces of bacteria contain AAT as a key component (Fig. 1d–f), along with branched GlcNAc or GalNAc residues. For example, AAT is present in the lipopolysaccharide of *Shigella sonnei*⁹, on the capsules of *Streptococcus pneumoniae*¹⁰ and *B. fragilis*¹¹, on the cell wall polysaccharides of *S. mitis*¹², *S. pneumoniae*¹³ and *Providencia alcalifaciens*¹⁴, and in the repeating unit of *Bacillus cereus*¹⁵. Vaccine candidates against these bacteria are very much

in demand. However, the rare sugars are not accessible in acceptable purity and amounts by isolation. Availability of orthogonally protected monosaccharide building blocks through efficient chemical synthesis is the crucial step toward the development of glycoconjugate vaccines.

Over past few years, intense efforts have been devoted for the development of efficient protocols to access regioselectively protected monosaccharide building blocks that can be used as glycosyl donors or acceptors in complex glycan assembly, via regioselective deprotection or protection of sugars^{16–18}. Recently, two notable approaches were reported for regioselective monodeprotection of fully protected sugars. Palomo and co-workers¹⁹ used a regioselective deacetylation of peracetylated monosaccharides and disaccharides via enzymatic or chemoenzymatic strategies. On the other hand, Iadonisi and colleagues' protocol²⁰ is based on a regioselective mono-debenzylation of fully benzylated sugars by using Et₃SiH and I₂ reagent combination. These routes allow rapid synthesis of a variety of glycosyl acceptors containing acetyl or benzyl groups. Various groups are engaged in devising strategies for regioselective protection of sugars. Through their pioneering approach, Hung and co-workers established an elegant, regioselective one-pot orthogonal protection strategy for D-glucose^{21,22}, D-galactose²³, D-glucosamine²⁴ and D-mannose²⁵ hexopyranosides, by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a single catalyst, to access both selectively protected glycosyl donors and acceptors for one-pot glycosylation²⁶. Subsequently, several other groups reported regioselective and catalytic one-pot procedures for this purpose by using diverse routes^{27–32}. However, a general protocol for the synthesis of orthogonally protected building blocks of D-galactosamine and the bacterial rare sugars is still missing. This is mainly

Figure 1 | Structures of various bacterial glycans containing deoxy amino sugars.

because the rare sugars are not available by isolation, and because D-galactosamine is very expensive.

A few routes are reported for the synthesis of the bacterial rare sugars. Many of these monosaccharide building blocks can be synthesized by traditional carbohydrate strategies^{33–39}. However, those approaches mostly involve lengthy multistep routes with extensive protecting-group manipulations, tedious workup and chromatographic purifications and/or expensive starting materials. A *de novo* strategy was recently developed for AAT and other related deoxy amino sugars from L-threonine^{40,41} and L-Garner aldehyde⁴², respectively. These conceptually novel routes also entail a number of steps and intermittent separation of diastereomers.

We were interested in developing a short and general route for accessing all the rare-sugar and D-glycosamine building blocks. Toward this goal, we first developed a highly efficient, stepwise, double C2,C4 relay inversion procedure to access D-glucosamine and D-galactosamine building blocks from D-thiomannoside⁴³. Although the procedure offered a marked improvement over the existing ones, it still needed improvement, especially for the D-galactosamine building blocks, which entailed eight steps and three column-chromatographic purifications. We envisioned that a common intermediate, derived from an abundant and cheaply available natural monosaccharide such as D-mannose, could be efficiently transformed into various rare-sugar and

galactosamine building blocks, by carrying out regioselective and one-pot reactions. Such an approach would cut down the number of steps and obviate the workup and chromatographic purifications. Here we present an expedient protocol for the synthesis of various orthogonally protected, bacterial rare-sugar⁴⁴ and D-galactosamine⁴⁵ building blocks. Our strategy involves a one-pot double-serial and double-parallel displacement of the D-mannosyl or D-rhamnosyl 2,4-bis-trifluoromethanesulfonates (OTf, triflate) by azide, phthalimide, acetate and nitrite ions as nucleophiles. The D-rhamnose derivative can, in turn, be easily obtained from cheaply available D-mannose via a regioselective deoxygenation at C6.

Protocol development

Synthesis of orthogonally protected bacterial rare-sugar building blocks.

We selected the readily available β-D-thiomannoside **1** (ref. 46; Fig. 2) as the suitable starting material owing to its stereochemical relationship with the rare sugars. Compound **1** has the same stereochemistry at C3 and C5, and opposite stereochemistry at C2 and C4 as that of DATDH, AAT and FucNAc, whereas it has opposite stereochemistry at C2 alone in comparison with bacillosamine. The requisite D-rhamnose derivative **2** could be obtained from **1** by carrying out a regio-

selective deoxygenation at C6, followed by tin-mediated acylation at O3. We envisioned that 2,4-diol **2** could be manipulated by stereoinversions and/or a double inversion at C2 and/or C4 positions to rapidly access all the unusual sugars (**4**) via sequential, one-pot, double-parallel and double-serial inversions of the corresponding 2,4-bis-triflate derivative **3** by using azide, phthalimide, acetate and NO₂ anions as nucleophiles, if the desired regioselectivity could be achieved by adjusting the reaction conditions. It has been shown that the presence of the β-linkage in D-mannoside^{43,47} and the 3-O-acyl group^{43,48,49} is essential for successful nucleophilic displacements using azide or nitrite anions, respectively. We anticipated that the axial C2-OTf in β-D-rhamnose **2** would be more accessible than C4-OTf for attack by a nucleophile. Thus, selective

Figure 2 | A strategy to synthesize rare deoxy amino sugars via regioselective and one-pot nucleophilic displacements.

Figure 3 | Preparation of D-rhamnosyl 2,4-diols **2a/2b**.

azide displacement of C2-OTf, under controlled conditions, followed by sequential nucleophilic displacement of the C4-OTf by nitrite ion on β -D-rhamnose skeleton, seemed plausible.

To test this hypothesis, compound **1** (**Fig. 3**) was first regioselectively deoxygenated at C6 to obtain the corresponding C6-deoxy sugar (D-rhamnose^{50–56}), which upon a highly regioselective O3 acylation (ref. 57) afforded 2,4-diols **2a** and **2b**. These diols served as common intermediates to construct various rare-sugar building blocks⁴⁴. The reaction conditions and the results of regioselective nucleophilic displacements of **2a/2b** are summarized in **Figure 4** and **Table 1**. Our protocol involves the following: (i) triflation of **2a/2b** followed by S_N2 displacement of the formed bis-triflates by excess azide ions to form DATDH building blocks **5a/5b**; (ii) triflation of **2b** followed by a highly regioselective S_N2 displacement of the C2-OTf by azide ions and concomitant displacement of the C4-OTf by phthalimide ions to generate AAT building block **6**; (iii) triflation of **2b**, regioselective nucleophilic displacement of C2-OTf by azide, followed by S_N2 displacement of the C4-OTf by nitrite ions to generate 4-OH fucosamine derivative **7**; (iv) triflation of **2a**, nucleophilic displacement of C2-OTf by azide, followed by an internal S_N2 displacement of the C4-OTf by C3-OAc group to obtain 3-OH fucosamine derivative **8**; and (v) triflation of the 4-OH fucosamine **7** followed by S_N2 displacement of the C4-OTf by azide ions to access the bacillosamine building block **9**. The key to the success of this approach lies in the careful tuning of the reaction conditions to generate a single regioisomer in the first displacement of C2-OTf, which allows sequential addition of reagents to achieve the subsequent nucleophilic displacement of C4-OTf (azide, phthalimide or acetate) in an essentially one-pot manner.

Accordingly, as shown in **Figure 4**, diols **2a** and **2b** were individually converted into the 2,4-diazido derivatives (DATDH) **5a** (82%) and **5b** (85%), respectively, via a double-parallel S_N2 displacement of triflates by sodium azide (NaN_3) in an essentially one-pot manner.

For the double-serial inversion, diol **2b** was first converted into the corresponding 2,4-bis-triflate, which upon treatment with a stoichiometric amount of tetrabutylammonium azide (TBAN_3) in acetonitrile (CH_3CN) at -30°C smoothly underwent a highly regioselective displacement of the C-2 triflyloxy group (OTf), exclusively. The remaining C4-OTf was concomitantly displaced by excess potassium phthalimide to afford the AAT building block **6** in 57% overall yield. In this transformation, it was necessary to carry out a brief aqueous workup after the triflation, so as to

obtain the exact weight of the crude triflate and accordingly add an exact triflate equivalent of TBAN_3 to obtain a single product. The regioselectivity observed in this case can be attributed to steric factors. It is anticipated that the C-2-OTf of the β -isomers **2a/2b** is more accessible as compared with the C-4-OTf. Further tuning of regioselectivity could be attained by using an exact stoichiometric amount of reagent and conducting the displacement at a low temperature. In general, for all the serial double inversions, we carried out a brief aqueous workup and vacuum drying of the crude product to find out the accurate weight of the crude product, whereas for all the double parallel inversions only evaporation of dichloromethane (DCM) was done before the addition of NaN_3 .

To access the D-fucosamine derivative, we used a Lattrell-Dax reaction^{58–60}, which involves nucleophilic displacement of triflate by using nitrite anions. Accordingly, triflation of compound **2b** and its concomitant treatment with TBAN_3 , as described earlier, was conducted first. After the completion of the reaction, tetrabutylammonium nitrite (TBANO_2) was added in the same pot to displace the 4-OTf and generate the D-fucosamine 4-OH derivative **7** (60%) exclusively. We used a different strategy to access D-fucosamine derivative with a 3-OH free. Starting with the C3-acetate **2a**, we repeated the first two steps in a similar manner to obtain the corresponding 2-azido-4-OTf (quinovosamine) derivative. Then, we added a small amount of water into the same pot and the reaction was refluxed to generate 3-hydroxy fucosamine **8** (62%) as a single product. In this water-mediated transformation, the 3-O-acetyl group migrated from C3 to C4 along the top face of the sugar ring to displace the C4-triflyloxy group with inversion at C4 (ref. 61).

Finally, sequential triflation of **7** followed by concomitant azide displacement of C4-OTf afforded the bacillosamine building block **9** in an essentially one-pot manner (81%). In this way, all the bacterial rare sugar building blocks **5–9** could be conveniently prepared in 1–2 d, from a common intermediate **2a** or **2b** via

Figure 4 | Efficient synthesis of bacterial deoxy amino sugar thioglycoside building blocks.

TABLE 1 | Synthesis of bacterial rare-sugar building blocks.

Substrate	Nucleophile 1	Nucleophile 2	Product	Yield (%)
2a	NaN ₃	NaN ₃	5a	82
2b	NaN ₃	NaN ₃	5b	85
2b	TBAN ₃	PhthNK	6	57
2b	TBAN ₃	TBANO ₂	7	60
2a	TBAN ₃	OAc	8	62
7a	NaN ₃	—	9	81

^aIt involves only C4 OTf displacement by sodium azide.

regioselective one-pot reactions, after a single chromatographic purification in 57–85% overall yields (Table 1). Starting from cheaply available D-mannose, the entire process takes 4–5 d and involves three column-chromatographic purifications.

Synthesis of orthogonally protected D-galactosamine building blocks. By using this protocol, we transformed the mannosyl diols **10a/10b** into various orthogonally protected D-galactosamine thioglycoside building blocks in a similar manner⁴⁵. For this purpose, first compounds **10a** and **10b** were readily prepared from **1** (Fig. 5) via regioselective silylation at O6, followed by highly catalytic, tin-mediated acetylation or benzylation at O3 in excellent overall yields. The reaction conditions and the results of regioselective nucleophilic displacements of **10a/10b** are summarized in Figure 6 and Table 2. As shown in Figure 6, triflation of **10a/10b** followed by a double-parallel displacement of the corresponding 2,4-bis-triflates using NaN₃ in *N,N*-dimethyl formamide (DMF) afforded the 4-azido galactosamine derivatives **11a** and **11b** in 85% and 84% overall yields, respectively, after a single chromatographic purification, in an essentially one-pot manner. Similarly, the use of the double-serial inversion protocol, triflation of **10a** and **10b**, followed by a regioselective nucleophilic displacement of the C2-triflyloxy group at –30 °C by stoichiometric TBAN₃, and a sequential one-pot nitrite ion-mediated inversion at C4 using TBANO₂ (3.0 equivalents) generated orthogonally protected D-galactosamine derivatives **12a** and **12b**, in 60% and 61% overall yields, respectively. By swapping the order of the addition of the reagents (i.e., first adding 3.0 equivalents of TBANO₂ at 0 °C, then adding 3.0 equivalents of TBAN₃ at 0 °C and allowing it to reach room temperature (RT; 25–30 °C)), the 4-azido, 2-hydroxy galactose derivatives **13a** and **13b** were obtained from **10a** and **10b** in 54% and 56% overall yields, respectively (Box 1). Finally, the acetate migration protocol described earlier for **2a** worked well on

Figure 5 | Preparation of D-mannosyl 2,4-diols **10a/10b**.

10a to smoothly deliver the 3-hydroxy GalN₃ derivative **14** (56%) in a one-pot manner.

Thus, a complete set of orthogonally protected rare sugars, as well as D-galactosamine building blocks, was rapidly synthesized from D-mannose in a highly efficient manner (Tables 1 and 2). All the monosaccharide building blocks reported in this study are new compounds, which are thoroughly characterized by using ¹H, ¹³C and 2D NMR spectral analysis.

Synthesis of orthogonally protected D-glucosamine thioglycosides. A simple protocol for the preparation of orthogonally protected D-glucosamine building blocks from thiomannoside **1** is outlined in Figure 7 (Box 2; ref. 43). It involves (i) a highly regioselective O3 acylation of **1**, (ii) a rapid 4,6-O-benzylidenation and (iii) C2 epimerization via nucleophilic displacement of the corresponding C2-triflyloxy group. This stepwise procedure involving column chromatography at each step gives D-GlcN₃ building blocks **17a** and **17b** in 66% and 70% overall yields, respectively. Alternatively, the four-step sequence could be carried out on a 2-g scale in 1 d without any intermittent purification to obtain compounds **17a** and **17b** in 45% and 44% overall yields, respectively, after a single chromatographic purification. This route is particularly useful for obtaining glucosamine derivatives. Compound **17a/17b** can be elaborated into various other orthogonal D-glycosamine building blocks. The benzylidene acetal in **17a/17b** can be manipulated by regioselective reductive

Figure 6 | Efficient synthesis of D-galactosamine thioglycoside building blocks.

TABLE 2 | Synthesis of galactosamine building blocks.

Substrate	Nucleophile 1	Nucleophile 2	Product	Yield (%)
10a	NaN ₃	NaN ₃	11a	85
10b	NaN ₃	NaN ₃	11b	84
10a	TBAN ₃	TBANO ₂	12a	60
10b	TBAN ₃	TBANO ₂	12b	61
10a	TBANO ₂	TBAN ₃	13a	54
10b	TBANO ₂	TBAN ₃	13b	56
10a	TBAN ₃	OAc	14	56

O4 or O6 opening or via hydrolysis and selective O6 protection. Compounds **17a** and **17b** were also converted to galactosamine derivatives **12a** and **12b** via benzylidene hydrolysis, regioselective

O6 silylation and C4 epimerization under Lattrell-Dax conditions. However, this sequence is lengthy, and we recommend that the one-pot procedure described earlier be followed for this purpose.

Applications of the approach

The rare-sugar thioglycoside building blocks could be used as stable glycosyl donors or acceptors to stereoselectively assemble the bacterial glycoproteins and oligosaccharides. As an application of our methodology, we show herein a short synthesis of the disaccharide moiety of the ZPS A1 found in *B. fragilis* by using building blocks **6** and **12a** (Fig. 8; ref. 45). Thioglycoside **6** was treated with bromine to generate the corresponding glycosyl bromide, which was orthogonally glycosylated with the thioglycoside acceptor **12a** by using silver trifluoromethanesulfonate (AgOTf) as a promoter to obtain the key disaccharide moiety of *B. fragilis* **18** (81%, α -only). Orthogonally protected disaccharide **18** is suitable for elaboration into the tetrasaccharide

Box 1 | Synthesis of 4-azido galactose derivatives **13a** and **13b** ● TIMING ~15.5 h

The 4-azido galactose derivatives **13a** and **13b** can be synthesized in a one-pot manner by double-serial displacement of β -D-mannosyl 2,4-bis triflates by using TBANO₂ followed by TBAN₃.

1. Perform PROCEDURE Steps 2–6 (starting with 6.0 mmol of **10a** or **10b**).
2. Perform PROCEDURE Step 7B(i–v).
3. Add acetonitrile (12 ml) to the compound obtained from step 2 and cool the flask to 0 °C.
4. Add 18.0 mmol of TBANO₂ (3.0 equivalents) and keep the reaction mixture at the same temperature for 30 min.
5. After the complete consumption of the starting material (as monitored by TLC), add TBAN₃ (3.0 equivalents) and keep the reaction mixture for stirring at RT for 3 h.
6. Work up as described in PROCEDURE Step 7A(v–x).
7. Identify the fractions containing compound **13a** or **13b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) for **13a** (R_f 0.4) and **13b** (R_f 0.4). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
8. Collect the fractions and evaporate the solvent by using a rotary evaporator.
9. Dry the residue under vacuum to obtain compound **13a** (yield 54%) or **13b** (yield 56%) as a pale yellowish liquid.
- **PAUSE POINT** Compounds **13a** and **13b** can be stored at RT for several months without marked decomposition.
10. Characterize the product **13a** or **13b** from step 9 by NMR spectroscopy using deuterated chloroform as the solvent.

● TIMING

Synthesis of 4-azido galactose derivative **13a** and **13b**

Steps 1–2: 6.5 h

Steps 3–5: 4 h

Steps 6–10: 5 h

Total time for steps 1–10: 15.5 h; all steps should be performed in a single day.

ANALYTICAL DATA

13a TLC petroleum ether:ethyl acetate 9:1 (vol/vol) R_f 0.4

¹H NMR (400 MHz, CDCl₃) δ 7.67–7.65 (m, 4H, ArH), 7.50–7.38 (m, 9H, ArH), 7.29–7.27 (m, 2H, ArH), 5.07 (dd, J = 9.6, 3.4 Hz, 1H, H-3), 4.50 (d, J = 9.6 Hz, 1H, H-1), 4.19 (d, J = 3.4 Hz, 1H, H-4), 3.85–3.76 (m, 3H, H-2, H-6a & H-6b), 3.70 (t, J = 6.4 Hz, 1H, H-5), 2.19 (s, 3H, CH₃), 1.06 (s, 9H, (CH₃)₃CSi).

¹³C NMR (100 MHz, CDCl₃) δ 170.6, 135.7, 133.0, 132.9, 132.8, 131.6, 130.1, 129.2, 128.4, 128.0, 89.3, 77.28, 76.0, 67.5, 62.4, 60.3, 26.9, 20.9, 19.3.

HR-ESI-MS (m/z): [M + Na]⁺ calculated for C₃₀H₃₅N₃O₅NaSSi, 600.1964; found 600.1965.

13b TLC petroleum ether:ethyl acetate 9:1 (vol/vol) R_f 0.4

¹H NMR (400 MHz, CDCl₃) δ 8.14–8.12 (m, 2H, ArH), 7.68–7.58 (m, 5H, ArH), 7.52–7.36 (m, 10H, ArH), 7.30–7.27 (m, 3H, ArH), 5.40 (dd, J = 9.6, 3.6 Hz, 1H, H-3), 4.59 (d, J = 9.6 Hz, 1H, H-1), 4.28 (d, J = 3.6 Hz, 1H, H-4), 3.97 (t, J = 9.6 Hz, 1H, H-5), 3.89–3.77 (m, 3H, H-2, H-6a & H-6b), 1.06 (s, 9H, (CH₃)₃CSi).

¹³C NMR (100 MHz, CDCl₃) δ 166.2, 135.7, 133.8, 133.0, 132.8, 131.8, 130.3, 130.1, 129.2, 129.1, 128.7, 128.3, 128.0, 89.5, 77.4, 76.2, 68.0, 62.4, 60.8, 27.0, 19.3.

HR-ESI-MS (m/z): [M + Na]⁺ calculated for C₃₅H₃₇N₃O₅NaSSi, 662.2121; found 662.2158.

Figure 7 | Efficient synthesis of D-glucosamine thioglycoside building blocks.

repeating unit. It should be noted that both the building blocks **6** and **12a** are prepared from D-mannose by using the one-pot protocol. Conceptually, this is the first example in which such a

complex disaccharide is synthesized from a common and naturally abundant sugar such as D-mannose.

By using our protocol, we also accomplished the first synthesis of the α -L-serine-linked trisaccharide of *N. meningitidis* (Fig. 9; ref. 44). Here a major challenge was achieving α -stereoselectivity in coupling of the monosaccharide with the primary OH of the amino acid L-serine. One of the salient features of our method is that the building blocks are all stable thioglycosides, which can be stored at RT for months. Advantageously, they offer the flexibility to switch between various types of donors (e.g., glycosyl sulfoxides, halides, trichloroacetimidates), by selective anomeric modification so that separate synthesis of individual donors is spared. Of the various glycosyl donors tried, the trichloroacetimidate offered exclusive α -selectivity. First, the thioglycoside building block **5a** was transformed into trichloroacetimidate donor **19**,

Box 2 | Synthesis of orthogonally protected D-glucosamine building blocks

D-glucosamine building blocks can be conveniently prepared from β -D-thiomannoside **1** via a four-step sequence involving regioselective O3 acylation, 4,6-O-benzylidenation and C2 inversion via azide displacement of the C2-triflate.

ADDITIONAL MATERIALS

Benzaldehyde dimethyl acetal (Sigma-Aldrich, cat. no. 226076) ! CAUTION This compound is harmful if swallowed; it causes eye, respiratory and skin irritation. Do not breathe the vapor.

Camphor-10-sulfonic acid (Sigma-Aldrich, cat. no. 147923) ! CAUTION This compound causes severe skin burns and eye damage; wear protective gloves/protective clothing/eye protection/face protection.

PROCEDURE

Synthesis of 3-O-acyl-mannose derivatives **15a** and **15b** ● TIMING ~10 h

1. Weigh 7.5 mmol of **1** (1.0 equivalents) into a 50-ml round-bottom flask and add a Teflon-coated magnetic stir bar.
2. Dry the flask under vacuum for 1 h, fill it with nitrogen, remove it from vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
3. Add THF:H₂O (48 ml, 19:1) and start the magnetic stirrer.
4. To this solution, add DIPEA (15.0 mmol), Me₂SnCl₂ (0.37 mmol) and 1.1 equivalents of acetyl chloride for **15a** or benzoyl chloride for **15b**.
5. Keep the reaction mixture at RT for 2.5 h. After complete consumption of starting material (as indicated by TLC), add 25 ml of 3% (vol/vol) HCl to quench the reaction.
6. Extract with ethyl acetate as described in PROCEDURE Step 7A(v-vii).
7. Pack a chromatography column (4 cm i.d. × 25 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 3:7 (vol/vol).
8. Dissolve the crude product obtained from step 6 with 10 ml of ethyl acetate and add 1.5 g of silica gel. Evaporate the solvent on a rotary evaporator to obtain a free-flowing solid; load it on top of the silica gel bed.
9. Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 3:7 (vol/vol) and collect fractions of 20–30 ml.
10. Identify the fractions containing **15a** and **15b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol) (*R_f* 0.5). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
11. Collect the fractions and evaporate the solvent by using a rotary evaporator.
12. Dry the residue under vacuum to obtain **15a** (yield 90%, foam) and **15b** (yield 92%, white solid).
13. Characterize the products **15a** and **15b** from step 12 by NMR spectroscopy, using deuterated chloroform as the solvent.

Synthesis of benzylidene protected mannose derivatives **16a** and **16b** ● TIMING ~6 h

14. Weigh 1.8 mmol of **15a** or **15b** (1.0 equivalents) into a 25-ml round-bottom flask and add a Teflon-coated magnetic stir bar.
15. Dry the flask under vacuum for 1 h, fill it with nitrogen, remove it from vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
16. Add 7 ml of acetonitrile and keep the magnetic stirrer on. To this solution, add 0.25 equivalents of 10-camphor sulfonic acid. Check the pH of the solution; it should be between 1 and 2.

▲ **CRITICAL STEP** The pH of the reaction mixture should be acidic.

17. Add benzaldehyde dimethylacetal (1.2 equivalents) dropwise under N₂ atmosphere and keep for stirring at RT for 10 min. Next, quench the reaction mixture with triethylamine.

▲ **CRITICAL STEP** The reaction mixture should not be kept for a long time.

(continued)

Box 2 | (continued)

18. Evaporate the solvents on a rotary evaporator under reduced pressure.
19. Pack a chromatography column (2 cm i.d. × 15 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 7:3 (vol/vol).
20. Dissolve the crude product obtained from step 18 with 5 ml of ethyl acetate and add 0.5 g of silica gel. Evaporate the solvent on a rotary evaporator to obtain a free-flowing solid; load it on top of the silica gel bed.
21. Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 7:3 (vol/vol) and collect fractions of 20–30 mL.
22. Identify the fractions containing **16a** and **16b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate 3:2 (vol/vol) (R_f 0.5). Products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
23. Collect the fractions and evaporate the solvent by using a rotary evaporator.
24. Dry the residue under vacuum to obtain **16a** (yield 90%, solid) and **16b** (yield 90%, white solid).
25. Characterize the products **16a** and **16b** from step 24 by NMR spectroscopy, using deuterated acetone as the solvent.

Synthesis of glucosamine derivatives **17a** and **17b** ● TIMING ~12 h

26. Weigh 1.6 mmol of **16a** or **16b** (1.0 equivalents) into a 25-mL round-bottom flask and add a Teflon-coated magnetic stir bar.
27. Dry the flask under vacuum for 1 h, fill it with nitrogen, remove it from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
28. Add dry DCM (9 mL) by using a syringe and turn the magnetic stirrer on. Add trifluoromethanesulfonic anhydride (3.12 mmol) and cool the flask to $-0\text{ }^{\circ}\text{C}$.
29. Add 3.12 mmol of pyridine and keep the reaction mixture at the same temperature for 10 min.
30. After the complete consumption of starting material (as monitored by TLC, developing with hexane:ethyl acetate (8:2)), visualize the product by UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- ▲ **CRITICAL STEP** Ensure that the starting material is completely consumed; if not, keep the reaction mixture for an additional 10 min.
31. Perform a solvent extraction as described in PROCEDURE Step 7B(i, ii).
32. Remove the sodium sulfate and the DCM as described in Step 7A(vi, vii).
33. Keep the residue under vacuum for 2 h, and then fill the flask with nitrogen and cap it with a rubber septum.
34. Add DMF (3.5 mL) to the compound obtained from step 33 and turn the magnetic stirrer on.
35. To this solution, add NaN_3 (3.12 mmol) and keep the reaction mixture for stirring at RT for 1 h.
36. After complete consumption of starting material (as monitored by TLC, developing with hexane:ethyl acetate (8:2) and visualizing by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate), dilute the reaction mixture with ethyl acetate (15 mL).
37. Extract with ethyl acetate as described in PROCEDURE Step 7A(v–vii).
38. Purify by column chromatography as described in PROCEDURE Step 7A (viii–x).
39. Identify the fractions containing **17a** and **17b** by silica gel TLC, developed with a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) (R_f 0.4). The products are visualized by measuring UV absorbance at 254 nm or by spraying them with a CAM stain and subsequently heating them on a hot plate.
40. Collect the fractions and evaporate the solvent by using a rotary evaporator.
41. Dry the residue under vacuum to obtain **17a** (yield 82%, White solid) and **17b** (yield 85%, white solid).
- **PAUSE POINT** Compounds **13a** and **13b** can be stored at RT for several months without marked decomposition. Characterize the products **17a** and **17b** from step 41 by NMR spectroscopy, using deuterated chloroform as the solvent.

● TIMING

Synthesis of 3-*O*-acyl-mannose derivatives **15a** and **15b**

- Steps 1–5: 4 h
 Steps 6 and 7: 1 h
 Steps 8–13: 5 h
 Total time for steps 1–13: 10 h; all steps should be performed in a single day.

Synthesis of benzylidene protected mannose derivatives **16a** and **16b**

- Steps 14–17: 2 h
 Steps 18–22: 3 h
 Steps 23–25: 1 h
 Total time for steps 14–25: 6 h; all steps should be performed in a single day.

Synthesis of glucosamine derivatives **17a** and **17b**

- Steps 26–29: 2 h
 Steps 30–35: 5 h
 Steps 36–41: 5 h
 Total time for steps 26–41: 12 h; all steps should be performed in a single day.

(continued)

Box 2 | (continued)

ANALYTICAL DATA

15a *TLC petroleum ether:ethyl acetate 3:7 (vol/vol)* R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 7.42–7.39 (m, 2H, ArH), 7.23–7.19 (m, 3H, ArH), 4.94 (s, 1H, H-1), 4.83 (dd, J = 10.0, 3.0 Hz 1H, H-3), 4.30 (d, J = 3.0 Hz, 1H, H-2), 4.25 (t, J = 10.0 Hz, 1H, H-4), 3.93 (bs, 2H, H-6), 3.37–3.35 (m, 1H, H-5), 2.14 (s, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 171.4, 134.2, 131.0, 129.2, 127.6, 87.3, 80.3, 76.7, 71.0, 63.6, 61.2, 21.2.

HRMS calculated for $\text{C}_{14}\text{H}_{18}\text{O}_6\text{S}$ $[\text{M} + \text{Na}]^+$ 337.0722; found 337.0711.

15b *TLC petroleum ether:ethyl acetate 3:7 (vol/vol)* R_f 0.5

^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 8.08 (d, J = 7.0 Hz, 2H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 7.52–7.48 (m, 4H, ArH), 7.31 (t, J = 7.5 Hz, 2H, ArH), 7.23 (t, J = 7.5 Hz, 1H, ArH), 5.27 (s, 1H, H-1), 5.10 (dd, J = 9.8, 3.3 Hz, 1H, H-3), 4.43 (d, J = 3.3 Hz, 1H, H-2), 4.21 (t, J = 9.8 Hz, 1H, H-4), 3.92 (dd, J = 12.0, 2.5 Hz, 1H, H-6a), 3.81 (dd, J = 12.0, 5.1 Hz, 1H, H-6b), 3.60–3.56 (m, 1H, H-5).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) δ 166.7, 137.0, 133.8, 131.3, 130.5, 130.3, 129.8, 129.2, 127.3, 87.7, 82.0, 79.0, 71.4, 65.3, 62.5.

HRMS calculated for $\text{C}_{19}\text{H}_{20}\text{O}_6\text{S}$ $[\text{M} + \text{Na}]^+$ 399.0878; found 399.0880.

16a *TLC petroleum ether:ethyl acetate 7:3 (vol/vol)* R_f 0.5

^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 7.49–7.44 (m, 4H, ArH), 7.37–7.30 (m, 5H, ArH), 7.26–7.22 (m, 1H, ArH), 5.65 (s, 1H, benzylidene), 5.35 (s, 1H, H-1), 5.07 (dd, J = 10.0, 3.3 Hz 1H, H-3), 5.00 (dd, J = 5.7, 0.8 Hz, OH; exchangeable by D_2O), 4.43–4.38 (m, 1H, H-2), 4.27 (dd, J = 10.0, 5.0 Hz, 1H, H-6eq), 4.19 (t, J = 10.0 Hz, 1H, H-4), 3.84 (t, J = 10.0 Hz, 1H, H-6ax), 3.69 (dt, J = 10.0, 5.0 Hz, 1H, H-5), 2.03 (s, 3H, CH_3).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) δ 170.8, 138.9, 136.7, 130.4, 129.8, 129.6, 128.8, 127.5, 127.2, 102.4, 88.46, 76.3, 74.3, 71.9, 71.4, 69.0, 20.9.

HRMS calculated for $\text{C}_{21}\text{H}_{22}\text{O}_6\text{S}$ $[\text{M} + \text{Na}]^+$ 425.1035; found 425.1026.

16b *TLC petroleum ether:ethyl acetate 7:3 (vol/vol)* R_f 0.5

^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 8.08 (d, J = 7.2 Hz, 2H, ArH), 7.62 (t, J = 7.5 Hz, 1H, ArH), 7.52–7.41 (m, 6H, ArH), 7.35–7.23 (m, 6H, ArH), 5.72 (s, 1H, benzylidene), 5.46 (s, 1H, H-1), 5.40 (dd, J = 10.0, 3.3 Hz 1H, H-3), 4.58 (d, J = 3.3 Hz, 1H, H-2), 4.39 (t, J = 10.0 Hz, 1H, H-4), 4.31 (dd, J = 10.0, 5.0 Hz, 1H, H-6eq), 3.90 (t, J = 10.0 Hz, 1H, H-6ax), 3.79 (dt, J = 10.0, 5.0 Hz, 1H, H-5).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) δ 166.3, 138.9, 136.8, 134.0, 131.0 (2C), 130.5, 129.9, 129.6, 129.3, 128.8, 127.5, 127.1, 102.3, 88.67, 76.5, 75.0, 72.0, 71.6, 69.1.

HRMS calculated for $\text{C}_{26}\text{H}_{24}\text{O}_6\text{S}$ $[\text{M} + \text{Na}]^+$ 487.1191; found 487.1171.

17a *TLC petroleum ether:ethyl acetate 9:1 (vol/vol)* R_f 0.4

^1H NMR (400 MHz, CDCl_3) δ 7.59–7.55 (m, 2H, ArH), 7.42–7.33 (m, 8H, ArH), 5.48 (s, 1H, benzylidene), 5.24 (t, J = 10.0 Hz, 1H, H-3), 4.61 (d, J = 10.0 Hz, 1H, H-1), 4.39 (dd, J = 11.2, 4.4 Hz, 1H, H-6eq), 3.78 (t, J = 10.0 Hz, 1H, H-4), 3.55–4.52 (m, 2H, H-6a, H-5), 3.41 (t, J = 10.0 Hz, 1H, H-2), 2.11 (s, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 169.7, 136.8, 134.0, 130.7, 129.39, 129.33, 129.03, 128.4, 126.2, 101.6, 87.1, 78.4, 73.1, 70.8, 68.5, 63.7, 20.9.

HRMS calculated for $\text{C}_{21}\text{H}_{21}\text{O}_5\text{SN}_3$ $[\text{M} + \text{Na}]^+$ 450.1100; found 450.1079.

17b *TLC petroleum ether:ethyl acetate 9:1 (vol/vol)* R_f 0.4

^1H NMR (400 MHz, CDCl_3) δ 8.08 (d, J = 7.2 Hz, 2H, ArH), 7.62–7.56 (m, 3H, ArH), 7.40–7.27 (m, 10H, ArH), 5.50 (t, J = 9.6 Hz, 1H, H-3), 5.47 (s, 1H, benzylidene), 4.69 (d, J = 9.6 Hz, 1H, H-1), 4.39 (dd, J = 9.6, 5.0 Hz 1H, H-6eq), 3.80 (t, J = 9.6 Hz, 1H, H-6ax), 3.71 (t, J = 9.6 Hz, 1H, H-4), 3.63 (dt, J = 9.6, 4.4 Hz 1H, H-5), 3.58 (t, J = 9.6 Hz, 1H, H-2).

^{13}C NMR (100 MHz, CDCl_3) δ 165.4, 136.7, 133.8, 133.5, 130.8, 130.0, 129.4, 129.3, 129.1, 128.9, 128.5, 128.3, 126.2, 101.5, 87.3, 78.5, 73.6, 70.9, 68.5, 64.1.

HRMS calculated for $\text{C}_{26}\text{H}_{23}\text{O}_5\text{SN}_3$ $[\text{M} + \text{Na}]^+$ 512.1256; found 512.1264.

which was concomitantly coupled with the L-serine acceptor **20**, to cleanly generate a single α -isomer (92%), which upon selective removal of O3 acetate group (triethylamine and methanol) afforded glycosyl amino acid acceptor **21** (80%). Subsequent glycosylation of **21** with the di-galactosyl α -glycosyl chloride **22** (ref. 44) generated exclusively α -linked product **23** (80%), which upon global deprotection delivered the target molecule **24** in 51% overall yield. In a similar way, other rare-sugar building blocks can be used for synthesizing various bacterial glycans. The D-GalNAc and D-GlcNAc motifs are also present in several important glycoconjugates, including mucin type O-glycoproteins, N-glycoproteins, globo-H and ganglioside cancer antigens⁶². Synthesis of these glycosamine-containing glycoconjugates can

also be achieved through this protocol. Toward this end, we have used building block **14** for the synthesis of orthogonally protected T_N cancer antigen⁴⁵.

Bacterial glycoproteins and oligosaccharides are emerging as potential glycoconjugate vaccine candidates. Synthesis of antigenic vaccine candidates would be greatly facilitated by an expedient protocol. Ready availability of such orthogonally protected rare-sugar building blocks in conjunction with automated oligosaccharide synthesis⁶³ would expedite the bacterial glycan assembly. It would also provide structurally varied azido sugar intermediates that can be metabolically incorporated selectively into bacterial surface glycans in order to tag them further using bio-orthogonal probes for live-cell imaging^{64–66}.

PROTOCOL

Figure 8 | A short synthesis of the disaccharide moiety of the ZPS A1 from *B. fragilis*.

Experimental design

For the success of the regioselective one-pot reactions, the experimenter should remember that it is necessary to critically observe the temperature conditions so as to obtain the desired regioselectivity in the first displacement step during the double-serial protocol. The temperature of this step should not be raised higher than $-30\text{ }^{\circ}\text{C}$. The regioselectivity is lost at $-15\text{ }^{\circ}\text{C}$ and the corresponding diazido compound starts to form. One of the limitations of our method is that compound **2a** cannot be used successfully in place of **2b** for the preparation of AAT **6** and 4-OH fucosamine **7**, as the 3-OAc group in **2a** is prone to migration under these conditions. In contrast, diol **2b** cannot be used in place of **2a** for synthesizing the 3-OH fucosamine derivative **8** because the 3-OBz group does not migrate to the same extent as the acetate group. Together, the OBz and OAc groups form a complementary pair with useful implications. This migratory aptitude should be considered when planning a synthesis of a particular bacterial glycan.

Figure 9 | Application of the protocol. Total synthesis of the trisaccharide of *N. meningitidis*.

MATERIALS

REAGENTS

- ! CAUTION** Always wear eye protection, a lab apron and hand gloves. All the reactions should be carried out in a fume hood, using clean and defect-free glassware and under nitrogen atmosphere.
- p*-Toluenesulfonyl chloride (TsCl; Spectrochem, cat. no. 0120142) **! CAUTION** There is a risk of serious damage to the eyes; wear eye/face protection.
- Lithium aluminum hydride (Spectrochem, cat. no. 011208) **! CAUTION** This compound causes severe skin burns and eye damage; keep it away from any possible contact with water, because of violent reaction and possible flash fire; handle it under inert gas, protect it from moisture and wear protective gloves/protective clothing/eye protection/face protection.
- Acetyl chloride (Spectrochem, cat. no. 0101168) **! CAUTION** Acetyl chloride reacts violently with water; it is a highly flammable liquid, and it causes severe skin burns and eye damage; wear protective gloves/eye protection.
- Benzoyl chloride (Spectrochem, cat. no. 0102214) **! CAUTION** It is harmful if swallowed; it causes severe skin burns and eye damage and may cause an allergic skin reaction.

- Dimethyltin dichloride (Me_2SnCl_2 ; Sigma-Aldrich, cat. no. 288012) **! CAUTION** Dimethyltin dichloride may cause respiratory irritation, and it is very toxic to aquatic life; wear protective gloves/protective clothing/eye protection.
- N,N*-diisopropylethylamine (DIPEA; Spectrochem, cat. no. 0104261) **! CAUTION** DIPEA is a highly flammable liquid and vapor. It is toxic if swallowed, and it causes severe skin burns and eye damage; keep it away from sources of ignition.
- Chloroform (Merck, cat. no. 82226592001730) **! CAUTION** Chloroform is harmful if it is swallowed; it causes skin irritation, and it is suspected of causing cancer; wear suitable protective clothing and gloves.
- Sulfuric acid (H_2SO_4 ; Merck, cat. no. 61752790301730) **! CAUTION** H_2SO_4 causes severe skin burns and eye damage. Never add water to this product; wear protective gloves/protective clothing/eye protection/face protection.
- Trifluoromethanesulfonic anhydride (Spectrochem, cat. no. 0120199) **! CAUTION** This compound reacts violently with water, and it is harmful if swallowed; wear protective gloves/eye protection/face protection.

- Pyridine (Merck, cat. no. 82230125001730) **! CAUTION** Pyridine is highly flammable and reacts violently with water; it is harmful on inhalation, on contact with skin or if swallowed.
- Dichloromethane (DCM; Merck, cat. no. 60604990251730) (dried over CaH_2) **! CAUTION** DCM has limited evidence of a carcinogenic effect; do not breathe its vapor, avoid contact with skin and eyes and wear suitable protective clothing and gloves.
- Sodium azide (NaN_3 ; Merck, cat. no. 61824401001730) **! CAUTION** It is very toxic; exposure via skin contact can be very harmful.
- *N,N*-dimethyl formamide (DMF; Merck, cat. no. 61836005001730) **! CAUTION** DMF is a flammable liquid and vapor; it is harmful on contact with skin, and it causes serious eye irritation.
- Tetrabutylammonium azide (TBAN_3 ; Sigma-Aldrich, cat. no. 651664) **! CAUTION** This compound causes skin irritation; avoid its release to the environment.
- Acetonitrile (Merck, cat. no. 61848805001730) **! CAUTION** Acetonitrile is a highly flammable liquid and vapor; it is harmful if swallowed, on contact with skin or if inhaled.
- Phthalimide potassium salt (Sigma-Aldrich, cat. no. 160385) **! CAUTION** Phthalimide potassium salt causes skin irritation and serious eye irritation.
- Tetrabutylammonium nitrite (TBANO_2 ; Sigma-Aldrich, cat. no. 86884) **! CAUTION** TBANO_2 causes eye, respiratory system and skin irritation.
- *tert*-Butylchlorodiphenyl silane (TBDPSCl ; Spectrochem, cat. no. 0102208) **! CAUTION** TBDPSCl causes burns and respiratory irritation; wear suitable protective clothing, gloves and eye/face protection.
- Bromine (Merck, cat. no. 60194500201730) **! CAUTION** Bromine causes severe skin burns and eye damage. It is fatal if it is inhaled, and is very toxic to aquatic life; avoid its release to the environment. Wear protective gloves/protective clothing/eye protection/face protection and respiratory protection.
- *N*-Bromosuccinimide (Spectrochem, cat. no. 010230) **! CAUTION** *N*-Bromosuccinimide is harmful if swallowed; wear protective gloves.
- Tetrahydrofuran (THF; Spectrochem, cat. no. 0120176) **! CAUTION** THF is a highly flammable liquid and vapor, and it causes serious eye irritation.
- Trichloroacetonitrile (Spectrochem, cat. no. 0120238) **! CAUTION** Trichloroacetonitrile is toxic to aquatic life with long-lasting effects; avoid its release to the environment.
- 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU; Spectrochem, cat. no. 010438) **! CAUTION** DBU causes severe skin burns and eye damage; wear suitable protective clothing, gloves and eye/face protection.
- Trimethylsilyl trifluoromethanesulfonate (TMSOTf ; Sigma-Aldrich, cat. no. 91741) **! CAUTION** TMSOTf is flammable, causes burns and respiratory irritation; wear suitable protective clothing, gloves and eye/face protection.
- Molecular sieves, 3 Å (Sigma-Aldrich, cat. no. 233641) **! CAUTION** Molecular sieves cause eye, skin and respiratory irritation; wear suitable protective clothing and rinse eyes/skin immediately if contact occurs.
- Triethylamine (Merck, cat. no. 8083520100) **! CAUTION** This reagent is highly flammable, harmful on inhalation, on contact with skin or if swallowed and causes severe burns; keep it in a cool place, and keep it away from sources of ignition. Wear suitable protective clothing, gloves and eye/face protection.
- Methanol (Merck, cat. no. 60600825001730) **! CAUTION** Methanol is a highly flammable liquid and vapor; keep it away from heat/sparks/open flames/hot surfaces; it causes damage to organs.
- Silver trifluoromethanesulfonate (AgOTf ; Sigma-Aldrich, cat. no. 176435) **! CAUTION** AgOTf causes eye, respiratory and skin irritation; wear suitable protective clothing and gloves.
- 2,4,6-Trimethylpyridine (sym-collidine; Sigma-Aldrich, cat. no. 27690) **! CAUTION** 2,4,6-Trimethylpyridine is a flammable liquid, and it is harmful if swallowed or if inhaled; wear protective gloves.
- Triphenylphosphine (Spectrochem, cat. no. 0120102) **! CAUTION** Triphenylphosphine is harmful if swallowed; it may cause an allergic skin reaction, as well as damage to organs (Nervous system) through prolonged or repeated exposure if inhaled; wear protective gloves.
- Sodium bromate (Merck, cat. no. 8450660250) **! CAUTION** Sodium bromate may intensify fire. It is an oxidizer. It is harmful if swallowed and causes skin irritation; contact with combustible material may cause fire.
- Sodium dithionite (Fisher Scientific, cat. no. 7775146) **! CAUTION** Sodium dithionite is self-heating, and it may catch fire; contact with acids liberates toxic gases, so keep it away from heat, sparks and flames.
- Sodium methoxide (Spectrochem, cat. no. 011913) **! CAUTION** Sodium methoxide reacts violently with water; it causes severe skin burns and eye damage; it is self-heating and may catch fire; wear protective gloves/protective clothing/eye protection/face protection.
- Hydrochloric acid (HCl ; 35% (vol/vol), Merck, cat. no. 61752690251730) **! CAUTION** HCl is corrosive, causes severe burns and may cause respiratory irritation; wear safety goggles and gloves while handling HCl .
- Sodium sulfate (Merck, cat. no. 61752190511730)
- Sodium chloride (Merck, cat. no. 61751990201730)
- Sodium bicarbonate (Merck, cat. no. 61752090501730)
- Ethyl acetate (Merck, cat. no. 61757092001730) **! CAUTION** Ethyl acetate is highly flammable; it causes eye irritation; repeated exposure may cause skin dryness or cracking, and vapors may cause drowsiness and dizziness; keep it away from sources of ignition.
- Toluene (Merck, cat. no. 61836605001730) **! CAUTION** Toluene is a highly flammable liquid; keep it away from heat/sparks/open flames/hot surfaces.
- Petroleum ether (Merck, cat. no. 61782225001730) **! CAUTION** Petroleum ether is highly flammable. It causes skin irritation. It is harmful and may cause serious damage to health by prolonged exposure through inhalation. It is toxic to aquatic organisms and may cause long-term adverse effects in the aquatic environment; there is also a possible risk of impaired fertility.
- Cerium(IV) sulfate ($(\text{Ce}(\text{SO}_4)_2)$; Sigma-Aldrich, cat. no. 359009)
- Ammonium molybdate tetrahydrate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$; Merck, cat. no. 60118201001730)

EQUIPMENT

- Round-bottomed flask (Borosil)
- Vacuum pump (Hindhivac)
- Funnel (Borosil)
- Filter papers (Asco)
- Separatory funnel (Borosil)
- UV lamp (Superfit India)
- Rotary evaporator (Büchi)
- Silica gel 60 F254 thin-layer chromatography (TLC) plates, 20 cm $\times$ 20 cm (Merck, cat. no. 105715)
- NMR tube (Norell)
- NMR spectrometer (Bruker, AV-400)
- Mass spectrometer (Bruker-QTOF)
- Teflon-coated magnetic stir bars (Sigma-Aldrich)
- Septum (Sigma-Aldrich)
- Glass syringe (Glass van)
- Micro-volume syringe (Hamilton)
- Ultra-low temperature chiller (Julabo)
- Dewar flask (Scam)
- Glass oven (Büchi)
- Hot plate magnetic stirrer (Heidolph)
- Chromatography column (Scam)
- Addition funnel (Scam)
- Conical flasks, 25-ml volume (Scam)
- Vacuum line and nitrogen source (Scam)

PROCEDURE

1| There are two starting materials that can be used for this main protocol: option A describes the synthesis of compounds **2a** and **2b**, and option B describes the synthesis of compounds **10a** and **10b**. The synthesis of **15a**, **15b** and the subsequent reactions using this starting material are described in **Box 2**.

(A) Synthesis of **2a** or **2b** ● **TIMING 30.5 h**

- (i) Weigh 35.0 mmol of phenyl-1-thio- β -D-mannopyranoside **1** (1.0 equivalents) into a 250-ml round-bottom flask and add a Teflon-coated magnetic stir bar.

- (ii) Dry the compound under vacuum for 1 h. Fill the flask with nitrogen, remove the flask from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
- (iii) Add pyridine (80 ml) with a syringe and cool the flask to 0 °C. To this, add a solution of TsCl (38.6 mmol, 1.1 equivalents) in pyridine (54 ml) dropwise by using an addition funnel. After the addition, remove the ice bath.
- (iv) Keep the reaction mixture at RT for 6 h. After the complete consumption of starting material (as indicated by TLC), remove the solvents under reduced pressure by using a rotary evaporator at a water bath temperature of 45 °C. This should take about 30–45 min.
- (v) Dissolve the residue obtained from Step 1A(iv) in 80 ml of chloroform and transfer it to a separatory funnel. Wash the organic phase with brine (20 ml). Separate the organic layer and dry it by adding about 1 g of sodium sulfate, shaking it mildly for 30 s and allowing it to stand for about 10 min.
- (vi) Filter the mixture through a filter paper on a funnel to remove the sodium sulfate and collect the filtrate in a 100-ml round-bottom flask.
- (vii) Evaporate the solvent by using a rotary evaporator at ~35 °C under vacuum (0–10 mbar). Dry the compound under high vacuum for 2 h.
- (viii) Weigh 100 mmol of lithium aluminum hydride under nitrogen atmosphere into a 500-ml round-bottom flask, add a Teflon-coated magnetic stir bar and cap the flask with a rubber septum. Add 60 ml of dry THF and cool the flask to 0 °C.
- (ix) Dissolve the compound obtained from Step 1A(vii) in 150 ml of THF and cannulate it dropwise into the cold solution prepared in Step 1A(viii).
- (x) Remove the ice bath, immerse the reaction flask into a preheated oil bath at 80 °C and reflux the reaction mixture for 3 h.
- (xi) After 3 h, cool the reaction mixture to 0 °C and quench the excess lithium aluminum hydride with the slow addition of ethyl acetate followed by a few drops of water.
! CAUTION This is an exothermic reaction; add the compound slowly to lithium aluminum hydride.
- (xii) Add 100 ml of 2N H₂SO₄ and transfer it into a separatory funnel. Wash the aqueous layer with ethyl acetate (100 ml × 3). Combine the organic phase and dry it over anhydrous sodium sulfate (2 g) for 10 min.
▲ CRITICAL STEP Check the final organic phase by TLC and ensure that no compound is present in the aqueous layer.
- (xiii) Filter the mixture through a filter paper on a funnel to remove the sodium sulfate and collect the filtrate in a 250-ml round-bottom flask.
- (xiv) Evaporate the solvent by using a rotary evaporator at ~35 °C under vacuum (0–10 mbar).
- (xv) Pack a chromatography column (4-cm internal diameter (i.d.) × 30 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 2:3 (vol/vol).
- (xvi) Dissolve the crude product obtained from Step 1A(xiv) with 10 ml of DCM and add 3 g of silica gel. Evaporate the solvent on a rotary evaporator to obtain a free-flowing solid, and then load it on top of the silica gel bed.
- (xvii) Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 2:3 (vol/vol); collect fractions of 30–40 ml.
- (xviii) Identify the fractions containing phenyl 6-deoxy-1-thio-β-D-mannopyranoside derivative by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol) (R_f 0.35). The products are visualized by measuring UV absorbance at 254 nm or by spraying with ceric ammonium molybdate (CAM) stain and subsequently heating on a hot plate. The CAM stain stock solution is prepared by mixing Ce(SO₄)₂ (5 g), (NH₄)₆Mo₇O₂₄ (12.5 g) and H₂SO₄ (50 ml) in distilled water (450 ml) with constant stirring.
- (xiv) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (xx) Dry the residue under vacuum to obtain phenyl 6-deoxy-1-thio-β-D-mannopyranoside derivative (yield 60%) as a white solid.
- (xxi) Characterize the product phenyl 6-deoxy-1-thio-β-D-mannopyranoside derivative from Step 1A(xx) by NMR spectroscopy by using deuterated acetone as a solvent.
- (xxii) Weigh 7.8 mmol of phenyl 6-deoxy-1-thio-β-D-mannopyranoside obtained from Step 1A(xx) (1.0 equivalents) into a 100-ml round-bottom flask, add a Teflon-coated magnetic stir bar and cap the flask with a rubber septum. Equip the flask with a nitrogen balloon.
- (xxiii) Add 40 ml of THF and turn on the magnetic stirrer.
- (xxiv) To this solution, add DIPEA (15.6 mmol), Me₂SnCl₂ (0.39 mmol) and 1.1 equivalents of acetyl chloride for **2a** or benzoyl chloride for **2b**.
- (xxv) Keep the reaction mixture at RT for 1 h. After complete consumption of starting material (as indicated by TLC), add 20 ml of 3% (vol/vol) HCl to quench the reaction.
- (xxvi) Transfer the solution into a separatory funnel and wash the aqueous layer with ethyl acetate (50 ml × 2). Combine the organic phase and dry it over anhydrous sodium sulfate (1.5 g) for 10 min.
- (xxvii) Perform Step 1A(xiii, xiv).

- (xxviii) Pack a chromatography column (3 cm i.d. × 25 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol).
- (xxix) Dissolve the crude product obtained from Step 1A(xxvii) with 10 ml of DCM, and add 2 g of silica gel. Evaporate the solvent on a rotary evaporator to obtain a free-flowing solid, and then load it on top of the silica gel bed.
- (xxx) Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol), and collect fractions of 20–30 ml.
- (xxxi) Identify the fractions containing **2a** and **2b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol) (R_f 0.35). The products are visualized by UV absorbance at 254 nm or by spraying with a CAM stain and subsequent heating on a hot plate.
- (xxxii) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (xxxiii) Dry the residue under vacuum to obtain **2a** (yield 89%, white solid) and **2b** (yield 92%, white solid).
■ PAUSE POINT Compounds **2a** and **2b** can be stored at RT for several months without marked decomposition.
- (xxxiv) Characterize the products **2a** and **2b** from Step 1A(xxxiii) by NMR spectroscopy, using deuterated chloroform as the solvent.

(B) Synthesis of **10a** or **10b** ● TIMING 21.5 h

- (i) Weigh 4.5 mmol of **1** (1.0 equivalents) into a 50-ml round-bottom flask; add a Teflon-coated magnetic stir bar.
- (ii) Dry the compound under vacuum for 1 h. Fill the flask with nitrogen and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
- (iii) Add pyridine (5 ml) with a syringe. After 5 min, add TBDPSCl (9.1 mmol, 1.1 equivalents) with a syringe and keep the reaction mixture at RT for 12 h.
■ PAUSE POINT The reaction mixture can be left at this temperature overnight.
- (iv) After the complete consumption of the starting material (as indicated by TLC), remove the solvents under reduced pressure by using a rotary evaporator.
- (v) Dissolve the residue obtained from Step 1B(iv) in 40 ml of chloroform and transfer it into a separatory funnel. Wash the organic phase with brine (20 ml). Separate the organic layer and dry it over sodium sulfate (1.0 g) for 10 min.
- (vi) Filter the organic layer through a filter paper on a funnel to remove sodium sulfate; collect the filtrate in a 100-ml round-bottom flask.
- (vii) Evaporate the solvent by using a rotary evaporator at ~35 °C under vacuum (0–10 mbar). Dry the compound under high vacuum for 1 h.
- (viii) Add THF (40 ml) to the compound obtained from Step 1B(vii), add a Teflon-coated magnetic stir bar and cap the flask with a rubber septum. Equip the flask with a nitrogen balloon.
- (ix) To this solution, add DIPEA (9.1 mmol), Me_2SnCl_2 (0.2 mmol) and 1.1 equivalents of acetyl chloride for **10a** or benzoyl chloride for **10b**.
- (x) Keep the reaction mixture at RT for 1 h. After complete consumption of starting material (as indicated by TLC), add 15 ml of 3% (vol/vol) HCl to quench the reaction and 50 ml of ethyl acetate. Separate the organic layer and add 1.0 g of sodium sulfate.
- (xi) Perform Step 1B(vi, vii).
- (xii) Pack a chromatography column (3 cm i.d. × 25 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol).
- (xiii) Dissolve the crude product obtained from Step 1B(xi) with 10 ml of DCM, and add 1 g of silica gel. Evaporate the solvent on a rotary evaporator to obtain a free-flowing solid, and load it on top of the silica gel bed.
- (xiv) Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol), and collect fractions of 20–30 ml.
- (xv) Identify the fractions containing **10a** and **10b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 3:2 (vol/vol) (R_f 0.5). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- (xvi) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (xvii) Dry the residue under vacuum to obtain **10a** (yield 84%, foam) and **10b** (yield 87%, white solid).
- (xviii) Characterize the products **10a** and **10b** from Step 1B(xvii) by NMR spectroscopy by using deuterated chloroform as the solvent.

Triflation ● TIMING ~3.5 h

- 2| Weigh 6.0 mmol of **2a** or **2b** (1.0 equivalents) (or **10a** or **10b**) into a 100-ml oven-dried round-bottom flask and add a Teflon-coated magnetic stir bar into it.

PROTOCOL

3| Dry the flask under vacuum for 1 h, fill it with nitrogen, remove it from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.

4| Add dry DCM (55 ml) by using a syringe, and turn the magnetic stirrer on. Cool the flask to $-10\text{ }^{\circ}\text{C}$ by using an ice-salt bath.

5| Add 78.4 mmol of pyridine (13.0 equivalents), and after 5 min add 36.9 mmol of trifluoromethanesulfonic anhydride (6.0 equivalents).

6| Allow the temperature of the ice bath to reach $10\text{ }^{\circ}\text{C}$ gradually over 2 h. After the starting material **2a** or **2b** (or **10a** or **10b**) is consumed (as monitored by TLC, developing with hexane:ethyl acetate (8:2)), visualize the product by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.

▲ CRITICAL STEP Ensure that the starting material is completely consumed; if not, keep the reaction mixture at $10\text{ }^{\circ}\text{C}$ for an additional 20 min.

? TROUBLESHOOTING

7| There are two options at this stage. Both options assume that the triflation reaction (Steps 2–6) has been performed. Option A describes the synthesis of **5a** and **5b** from the corresponding triflates derived from compounds **2a** and **2b**. The same steps can be used to prepare the 4-azido galactosamine derivatives **11a** and **11b** from the triflates derived from **10a** and **10b**. Option B describes the steps common to the synthesis of compounds **6** (from triflate of **2b**), **7** (from triflate of **2b**) and **8** (from triflate of **2a**). The same steps can be used for the synthesis of the GalNAc-4-OH derivatives **12a** and **12b** from **10a** and **10b**. If you are performing the latter synthesis, you should continue with Step 8B. GalNAc-3-OH derivative **14** can be prepared by carrying out Step 7B followed by Step 8C, using compound **10a** as a starting material.

(A) Synthesis of DATDH derivatives **5a** and **5b** ● TIMING ~18 h

- Evaporate the solvents by using a rotary evaporator at $\sim 35\text{ }^{\circ}\text{C}$. Dry the compound under vacuum for 1 h.
- Fill the flask with nitrogen and remove it from vacuum manifold. Cap the flask with a rubber septum and equip it with a nitrogen balloon. Add DMF (40 ml) and turn the magnetic stirrer on.
- Cool the flask to $0\text{ }^{\circ}\text{C}$ and add 60.0 mmol of NaN_3 (10 equivalents). After 10 min, remove the ice bath and continue stirring for 8 h at RT.
! CAUTION Gas may liberate vigorously during the process. Add NaN_3 portionwise and slowly.
■ PAUSE POINT The reaction mixture can be left at this temperature overnight.
- After the complete consumption of the starting material (as monitored by TLC), remove the nitrogen balloon and turn off the magnetic stirrer.
- Add ethyl acetate (50 ml) and water (40 ml) to the reaction mixture and transfer the mixture to a separatory funnel. Extract the aqueous layer with ethyl acetate (50 ml $\times$ 2). Combine the organic phase and dry it over anhydrous sodium sulfate (1 g) for 15 min.
- Filter the mixture through a filter paper on a funnel to remove the sodium sulfate; collect the filtrate in a 100-ml round-bottom flask.
- Evaporate the solvent by using a rotary evaporator at $\sim 35\text{ }^{\circ}\text{C}$ under vacuum (0–10 mbar).
- Pack a chromatography column (2.5 cm i.d. $\times$ 25 cm length) with silica gel by using a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) for **5a** and **5b**.
- Dissolve the crude product obtained from Step 7A(vii) with 5 ml of DCM, and add 1 g of silica gel. Evaporate the solvent on the rotary evaporator to obtain a free-flowing solid, and then load it on the top of the silica gel bed.
- Elute the product with a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) for **5a** and **5b**, and collect fractions of 20–25 ml.
- Identify the fractions containing compound **5a** or **5b** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) for **2a** (R_f 0.4) and **2b** (R_f 0.45). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- Collect the fractions and evaporate the solvent by using a rotary evaporator.
- Dry the residue under vacuum to obtain compound **5a** (yield 82%) or **5b** (yield 85%) as a pale yellowish liquid.
■ PAUSE POINT Compounds **5a** and **5b** can be stored at RT for several months without marked decomposition.
- Characterize the product **5a** or **5b** from Step 7A(xii) by NMR spectroscopy, using deuterated chloroform as the solvent.

(B) Synthesis of 2-azido-4-OTf (quinovosamine) derivative ● TIMING ~23 h

- Dilute the reaction mixture with 40 ml of DCM and transfer it into a separatory funnel.
- Wash the organic layer successively with 20 ml of 1 M HCl, 20 ml of aqueous NaHCO_3 and 20 ml of brine solution. Separate the organic layer and dry it over anhydrous sodium sulfate (1 g) for 15 min.

- (iii) Remove the sodium sulfate and the ethyl acetate as described in Step 7A(vi, vii).
- (iv) Dry the residue under vacuum for 2 h.
- (v) Fill the flask with nitrogen, remove it from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
- (vi) Add 160 ml of acetonitrile to the flask and cool the flask to $-30\text{ }^{\circ}\text{C}$ by using a cryostat. Turn the magnetic stirrer on.
- (vii) Add 6.0 mmol of TBAN_3 (1.0 equivalents) and keep the reaction mixture at the same temperature for 20 h.

▲ **CRITICAL STEP** Ensure that the starting material is completely consumed; if not, keep the reaction mixture for an additional 1 h.

? **TROUBLESHOOTING**

■ **PAUSE POINT** The reaction mixture can be left at this temperature overnight.

8| At this point, there are three options: preparation of compound **6** (option A), compound **7** (option B) or compound **8** (option C).

(A) Synthesis of AAT building block 6

- (i) After the complete consumption of the starting material (as monitored by TLC), remove the nitrogen balloon and turn off the magnetic stirrer. Evaporate the solvent under reduced pressure by using a rotary evaporator.
- (ii) Release the flask from the rotary evaporator under nitrogen atmosphere and cap it with a rubber septum. Equip the flask with a nitrogen balloon.
- (iii) Add DMF (30 ml) and turn the magnetic stirrer on.
- (iv) Add 12.0 mmol of phthalimide potassium salt (2.0 equivalents) and keep the reaction mixture at RT for 10 h.
■ **PAUSE POINT** The reaction mixture can be left at this temperature overnight.
- (v) Work up as described in Step 7A(v–x), by using a mixture of toluene and ethyl acetate 9:1 (vol/vol) as the solvent system for packing the column and eluting the product.
- (vi) Identify the fractions containing compound **6** by silica gel TLC developed with a mixture of toluene and ethyl acetate 9:1 (vol/vol). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- (vii) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (viii) Dry the residue under vacuum to obtain compound **6** (yield 57%) as a viscous liquid.
■ **PAUSE POINT** Compound **6** can be stored at RT for several months without marked decomposition.
- (ix) Characterize the product **6** from Step 8A(viii) by NMR spectroscopy by using deuterated chloroform as the solvent.

(B) Synthesis of FucNAc-4-OH derivative 7

- (i) After the complete consumption of the starting material (as monitored by TLC), add 18.0 mmol of TBANO_2 (3.0 equivalents) and keep the reaction mixture at RT for 1.5 h.
▲ **CRITICAL STEP** Ensure that the starting material is completely consumed; if not, keep the reaction mixture at RT for an additional 30 min.
? **TROUBLESHOOTING**
- (ii) Add ethyl acetate (50 ml) and brine (40 ml) to the reaction mixture and transfer it to a separatory funnel. Extract the aqueous layer with ethyl acetate (50 ml $\times$ 2). Combine the organic phase and dry it over anhydrous sodium sulfate (1.5 g) for 15 min.
- (iii) Work up as described in Step 7A(vi–x).
- (iv) Identify the fractions containing compound **7** by silica gel TLC, developed with a mixture of petroleum ether and ethyl acetate at a ratio of 8:2 (vol/vol) (R_f 0.3). The products are visualized by UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- (v) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (vi) Dry the residue under vacuum to obtain compound **7** (yield 60%) as a pale yellowish liquid.
- (vii) Characterize the product **7** from Step 8B(vi) by NMR spectroscopy, using deuterated chloroform as a solvent.

(C) Synthesis of FucNAc-3-OH derivative 8

- (i) After the complete consumption of the starting material (as monitored by TLC), reduce the volume of acetonitrile to one-third of its original volume.
- (ii) Add 22.0 equivalents of water; keep the reaction mixture for reflux at $65\text{ }^{\circ}\text{C}$ for 1.5 h.
▲ **CRITICAL STEP** Ensure that the starting material is completely consumed; if not, keep the reaction mixture at $65\text{ }^{\circ}\text{C}$ for an additional 30 min.
? **TROUBLESHOOTING**
- (iii) Add ethyl acetate (50 ml) and brine (40 ml) to the reaction mixture and transfer it to a separatory funnel. Extract the aqueous layer with ethyl acetate (50 ml $\times$ 2). Combine the organic phase and dry it over anhydrous sodium sulfate (1.5 g) for 10 min.

PROTOCOL

- (iv) Remove the sodium sulfate and the ethyl acetate as described in Step 7A(vi, vii).
- (v) Purify by column chromatography as described in Step 7A(viii–x).
- (vi) Identify the fractions containing compound **8** by silica gel TLC developed with a mixture of petroleum ether and ethyl acetate at a ratio of 8:2 (vol/vol) (R_f 0.25). The products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.
- (vii) Collect the fractions and evaporate the solvent by using a rotary evaporator.
- (viii) Dry the residue under vacuum to obtain compound **8** (yield 62%) as a pale yellowish liquid.
 - **PAUSE POINT** Compound **8** can be stored at RT for several months without marked decomposition.
- (ix) Characterize the product **8** from Step 8C(viii) by NMR spectroscopy, using deuterated chloroform as a solvent.

Synthesis of bacillosamine building block **9** ● **TIMING 19.5 h**

▲ **CRITICAL** The starting material for this section is compound **7**.

9| Weigh 1.0 mmol (1.0 equivalents) of *D*-fucosamine derivative **7** into a 50-ml round-bottom flask and add a Teflon-coated magnetic stir bar.

10| Dry the flask under vacuum for 1 h, fill it with nitrogen, remove it from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon.

11| Add dry DCM (8 ml) by using a syringe and turn the magnetic stirrer on. Cool the flask to $-10\text{ }^{\circ}\text{C}$.

12| Add 6.0 mmol of pyridine (6.0 equivalents). After 5 min add 1.2 mmol of trifluoromethanesulfonic anhydride (1.2 equivalents).

13| Allow the temperature of the ice bath to gradually reach $10\text{ }^{\circ}\text{C}$ over 2 h. After starting material **7** is consumed (as monitored by TLC, developing with hexane:ethyl acetate (9:1)), visualize the product by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.

▲ **CRITICAL STEP** Ensure that the starting material is completely consumed; if not, keep the reaction mixture at $10\text{ }^{\circ}\text{C}$ for an additional 20 min.

14| Evaporate the solvents by using a rotary evaporator at $\sim 35\text{ }^{\circ}\text{C}$. Dry the compound under vacuum for 1 h.

15| Fill the flask with nitrogen, remove it from the vacuum manifold and cap it with a rubber septum. Equip the flask with a nitrogen balloon. Add DMF (10 ml) and turn the magnetic stirrer on.

16| Cool the flask to $0\text{ }^{\circ}\text{C}$ and add 6.0 mmol of NaN_3 . After 10 min, remove the ice bath and continue stirring for 8 h at RT.

■ **PAUSE POINT** The reaction mixture can be left at this temperature overnight.

17| After the complete consumption of the starting material (as monitored by TLC), remove the nitrogen balloon and turn off the magnetic stirrer.

18| Add ethyl acetate (30 ml) and water (10 ml) to the reaction mixture and transfer it to a separatory funnel. Extract the aqueous layer with ethyl acetate (30 ml $\times$ 2). Combine the organic phase and dry it over anhydrous sodium sulfate (0.5 g).

19| Perform Step 7A(vi–x).

20| Identify the fractions containing compound **9** by silica gel TLC, developed with a mixture of petroleum ether and ethyl acetate at a ratio of 9:1 (vol/vol) (R_f 0.5). Products are visualized by measuring UV absorbance at 254 nm or by spraying with a CAM stain and subsequently heating on a hot plate.

21| Collect the fractions and evaporate the solvent by using a rotary evaporator.

22| Dry the residue under vacuum to obtain compound **9** (yield 81%).

■ **PAUSE POINT** Compound **9** can be stored at RT for several months without marked decomposition.

23| Characterize the product **9** from Step 22 by NMR spectroscopy, using deuterated chloroform as the solvent.

? TROUBLESHOOTING

Troubleshooting advice can be found in **Table 3**.

TABLE 3 | Troubleshooting table.

Problem	Possible reason	Solution
Low yield of 5a , 5b , 6 , 7 , 8	Triflation of 2a and 2b is a moisture-sensitive reaction	Reaction must be done under dry conditions. Moisture must be strictly avoided
Low yield of 5a , 5b , 6 , 7 , 8	Trifluoromethanesulfonic anhydride reacts violently with water. The reagent goes bad upon keeping it for a long time	Use fresh Trifluoromethanesulfonic anhydride. The reagent is a colorless liquid; if a white precipitate is seen, replace the reagent
Low yield of 5a , 5b , 6 , 7 , 8	Temperature fluctuation during C2 OTf displacement	Temperature should be strictly controlled at $-30\text{ }^{\circ}\text{C}$

● TIMING

Synthesis of **2a** or **2b**

Step 1A(i–iii): 1.5 h

Step 1A(iv–vii): 8.5 h

Step 1A(viii–xi): 4.5 h

Step 1A(xii–xiv): 1 h

Step 1A(xv–xxi): 6 h

Step 1A(xxii–xxvii): 3 h

Step 1A(xxviii–xxxiv): 6 h

Total time for Step 1A: 30.5 h; should be performed in 2 d

Synthesis of **10a** or **10b**

Step 1B(i–iii): 13.5 h

Step 1B(iv–vii): 2 h

Step 1B(viii–xi): 2 h

Step 1B(xii–xviii): 4 h

Total time for Step 1B: 21.5 h; should be performed in a single day

Triflation

Steps 2–6: 3.5 h

Synthesis of DATDH derivatives **5a**, **5b** and synthesis of 4-azido galactosamine derivatives **11a** and **11b**

Step 7A(i–iii): 8.5 h

Step 7A(iv–vii): 2 h

Step 7A(viii–xiv): 4 h

Total time for Step 7A(i–xiv): 14.5 h; should be performed in a single day

Synthesis of 2-azido-4-OTf (quinovosamine) derivative

Step 7B(i–iv): 3 h

Step 7B(v–vii): 20 h

Synthesis of AAT building block **6**

Steps 2–6: 3.5 h

Step 8A(i–iv): 11 h

Step 8A(v): 5 h

Step 8A(vi–ix): 3 h

Total time for Steps 2–8A: 22.5 h; should be performed in 2 d

Synthesis of FucNAc-4-OH derivative **7** and synthesis of GalNAc-4-OH derivative **12a** and **12b**

Step 2–6: 3.5 h

Step 7B (i–iv): 3 h

Step 7B (v–vii): 20 h

Step 8B(i-iii): 5 h

Step 8B(iv-vii): 3 h

Total time for Steps 2–8B: 34.5 h; should be performed in 2 d

Synthesis of FucNAc-3-OH derivative 8 and synthesis of GalNAc-3-OH derivative 14

Steps 2–6: 3.5 h

Step 7B (i-iv): 3 h

Step 7B (v-vii): 20 h

Step 8C(i-v): 5 h

Step 8C(vi-ix): 4 h

Total time for Steps 2–8C: 35.5 h; should be performed in 2 d

Synthesis of bacillosamine building block 9

Steps 9–12: 1.5 h

Steps 13–16: 12 h

Steps 17–23: 6 h

Total time for Steps 9–23: 19.5 h; should be performed in a single day

ANTICIPATED RESULTS

This expedient protocol is expected to speed up the synthesis of various antigenic bacterial glycoconjugates and facilitate vaccine development and drug discovery.

ANALYTICAL DATA

Phenyl 6-deoxy-1-thio-β-D-mannopyranoside derivative. TLC (petroleum ether:ethyl acetate, 2:3 vol/vol) R_f 0.35

^1H NMR (400 MHz, $(\text{CD}_3)_2\text{CO}$) δ 7.45–7.43 (m, 2H, ArH), 7.31–7.28 (m, 2H, ArH), 7.22–7.18 (m, 1H, ArH), 5.04 (s, 1H, H-1), 4.20 (bs, 3H, OH), 4.09 (s, 1H), 3.58 (dd, J = 9.0, 3.2 Hz, 1H), 3.44 (t, J = 9.0 Hz, 1H), 3.40–3.34 (m, 1H), 1.29 (d, J = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{CO}$) δ 137.6, 130.0, 129.7, 127.0, 87.5, 77.0, 75.7, 73.8, 73.3, 18.4.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{12}\text{H}_{16}\text{O}_4\text{NaS}$, 279.0667; found, 279.0663.

2a. TLC (petroleum ether:ethyl acetate, 3:2 vol/vol) R_f 0.35

^1H NMR (400 MHz, CDCl_3) δ 7.48–7.44 (m, 2H, ArH), 7.31–7.24 (m, 3H, ArH), 4.88 (d, J = 0.8 Hz, 1H, H-1), 4.75 (dd, J = 9.6, 3.2 Hz 1H, H-3), 4.28 (dd, J = 3.2, 0.8 Hz, 1H, H-2), 3.71 (t, J = 9.6 Hz, 1H, H-4), 3.45–3.38 (m, 1H, H-5), 2.15 (s, 3H, CH_3), 1.39 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 171.5, 133.8, 131.6, 129.2, 127.8, 87.0, 77.0, 76.9, 70.1, 70.5, 21.3, 18.0.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{14}\text{H}_{18}\text{O}_5\text{NaS}$, 321.0773; found, 321.0776.

2b. TLC (petroleum ether:ethyl acetate, 3:2 vol/vol) R_f 0.35

^1H NMR (400 MHz, CDCl_3) δ 8.05–8.03 (m, 2H, ArH), 7.56–7.54 (m, 1H, ArH), 7.48–7.46 (m, 2H, ArH), 7.41–7.37 (m, 2H, ArH), 7.32–7.25 (m, 3H, ArH), 5.00 (dd, J = 9.6, 3.2 Hz, 1H, H-3), 4.93 (d, J = 0.8 Hz, 1H, H-1), 4.40 (dd, J = 3.2, 0.8 Hz, 1H, H-2), 3.86 (t, J = 9.6 Hz, 1H, H-4), 3.48–3.44 (m, 1H, H-5), 1.41 (d, J = 6.0 Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 166.8, 134.0, 133.7, 131.5, 130.0, 129.4, 129.2, 128.6, 127.8, 87.1, 77.6, 76.8, 71.1, 70.7, 18.1.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{19}\text{H}_{20}\text{O}_5\text{NaS}$, 383.0929; found, 383.0941.

10a. TLC (petroleum ether:ethyl acetate, 3:2 vol/vol) R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 7.71–7.68 (m, 5H, ArH), 7.48–7.35 (m, 10H, ArH), 4.91 (s, 1H, H-1), 4.84 (dd, J = 10.0, 3.2 Hz, 1H, H-3), 4.30–4.28 (m, 1H, H-2), 4.14–4.09 (m, 1H, H-4), 3.97 (d, J = 4.8 Hz, 2H, H-6a, H-6b), 3.50–3.45 (m, 1H, H-5), 2.79 (bs, 1H, OH), 2.25 (d, J = 6.2 Hz, 1H, OH), 2.18 (s, 3H, CH_3), 1.07 (s, 9H, $\text{Si}(\text{CH}_3)_3$).

^{13}C NMR (100 MHz, CDCl_3) δ 171.1, 135.9, 135.8, 135.7, 134.0, 132.9, 132.7, 131.5, 131.3, 130.1, 129.2, 128.0, 127.86, 127.81, 87.2, 79.7, 77.0, 70.7, 67.0, 64.9, 26.8, 21.3, 19.3.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{30}\text{H}_{36}\text{O}_6\text{NaSSi}$, 575.1900; found, 575.1860.

10b. TLC (petroleum ether:ethyl acetate, 3:2 vol/vol) R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 8.12–8.10 (m, 2H, ArH), 7.73–7.72 (m, 4H, ArH), 7.71–7.24 (m, 14H, ArH), 5.09 (dd, J = 9.8, 3.2 Hz, 1H, H-3), 5.00 (d, J = 0.8 Hz, 1H, H-1), 4.43–4.41 (m, 1H, H-2), 4.25 (dt, J = 9.8, 3.2 Hz, 1H, H-4), 4.05–3.98 (m, 2H, H-6), 3.59–3.54 (m, 1H, H-5), 2.89 (d, J = 3.2 Hz, 1H, OH), 2.41 (d, J = 6.4 Hz, 1H, OH), 1.08 (s, 9H, $\text{Si}(\text{CH}_3)_3$).

^{13}C NMR (100 MHz, CDCl_3) δ 166.6, 135.87, 135.0, 134.2, 133.7, 133.0, 132.9, 131.5, 130.1, 130.09, 129.6, 129.3, 128.7, 128.0, 127.8, 87.3, 79.7, 76.9, 70.9, 67.2, 64.9, 27.0, 19.4.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{35}\text{H}_{38}\text{O}_6\text{NaSSi}$, 637.2056; found, 637.2054.

5a. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.4

^1H NMR (400 MHz, CDCl_3) δ 7.60–7.58 (m, 2H, ArH), 7.34–7.33 (m, 3H, ArH), 4.90 (dd, J = 10.0, 3.4 Hz, 1H, H-3), 4.40 (d, J = 10.0 Hz, 1H, H-1), 3.82 (d, J = 3.4 Hz, 1H, H-4), 3.70–3.65 (m, 2H, H-2 & H-5), 2.16 (s, 3H, CH_3), 1.35 (d, J = 6.2 Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 170.1, 133.5, 131.2, 129.2, 128.6, 86.5, 75.5, 73.4, 63.0, 59.3, 20.7, 17.8.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{14}\text{H}_{16}\text{N}_6\text{O}_3\text{NaS}$, 371.0902; found, 371.0905.

5b. TLC (toluene:ethyl acetate, 9:1 vol/vol) R_f 0.45

^1H NMR (400 MHz, CDCl_3) δ 8.10–8.07 (m, 2H, ArH), 7.64–7.59 (m, 3H, ArH), 7.50–7.46 (m, 2H, ArH), 7.38–7.35 (m, 3H, ArH), 5.18 (dd, $J = 10.0, 3.5$ Hz, 1H, H-3), 4.49 (d, $J = 10.0$ Hz, 1H, H-1), 3.85 (dd, $J = 3.5, 1.0$ Hz, 1H, H-4), 3.80–3.77 (m, 2H, H-2 & H-5), 1.39 (d, $J = 6.3$ Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 165.6, 133.9, 133.4, 131.2, 130.1, 129.1, 128.7, 128.5, 128.4, 86.6, 75.8, 73.6, 63.1, 59.8, 17.8.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{19}\text{H}_{18}\text{N}_6\text{O}_3\text{NaS}$, 433.1059; found, 433.1039.

6. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 7.89–7.83 (m, 3H, ArH), 7.79 (s, 3H, ArH), 7.72–7.66 (m, 2H, ArH), 7.64–7.61 (m, 1H, ArH), 7.49–7.27 (m, 5H, ArH), 5.39 (dd, $J = 9.8, 7.0$ Hz, 1H, H-3), 5.04 (dd, $J = 7.0, 2.8$ Hz, 1H, H-4), 4.91 (t, $J = 9.8$ Hz, 1H, H-2), 4.72 (d, $J = 9.8$ Hz, 1H, H-1), 4.00 (qd, $J = 6.4, 2.8$ Hz, 1H, H-5), 1.20 (d, $J = 6.4$ Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 168.5, 165.2, 134.5, 133.7, 133.5, 132.5, 129.8, 129.1, 128.8, 128.6, 128.1, 123.8, 88.9, 73.6, 73.2, 62.1, 51.7, 17.1.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{27}\text{H}_{22}\text{N}_4\text{O}_5\text{NaS}$, 537.1209; found, 537.1134.

7. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.3

^1H NMR (400 MHz, CDCl_3) δ 8.07–8.04 (m, 2H, ArH), 7.64–7.57 (m, 3H, ArH), 7.47–7.37 (m, 2H, ArH), 7.36–7.34 (m, 3H, ArH), 4.99 (dd, $J = 10.0, 2.9$ Hz, 1H, H-3), 4.53 (d, $J = 10.0$ Hz, 1H, H-1), 4.01 (d, $J = 2.9$ Hz, 1H, H-4), 3.83 (t, $J = 10.0$ Hz, 1H, H-2), 3.77 (q, $J = 6.4$ Hz, 1H, H-5), 1.38 (d, $J = 6.4$ Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 165.8, 133.8, 133.5, 131.6, 130.0, 129.2, 128.7, 128.6, 86.6, 76.8, 74.8, 69.4, 59.9, 16.8.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{19}\text{H}_{19}\text{N}_3\text{O}_4\text{NaS}$, 408.0994; found, 408.0974.

8. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.25

^1H NMR (400 MHz, CDCl_3) δ 7.61–7.58 (m, 2H, ArH), 7.33–7.30 (m, 3H, ArH), 5.15 (d, $J = 3.2$ Hz, 1H, H-4), 4.45 (d, $J = 10.0$ Hz, 1H, H-1), 3.72–3.67 (m, 2H, H-3 & H-5), 3.49 (t, $J = 10.0$ Hz, 1H, H-2), 2.13 (s, 3H, CH_3), 1.38 (d, $J = 6.4$ Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 133.1, 131.9, 129.0, 128.3, 86.5, 73.3, 73.1, 72.1, 62.4, 20.9, 16.9.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_4\text{NaS}$, 346.0837; found, 346.0819.

9. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.45

^1H NMR (400 MHz, CDCl_3) δ 8.08–8.06 (m, 2H, ArH), 7.63–7.59 (m, 3H, ArH), 7.49–7.45 (m, 2H, ArH), 7.38–7.35 (m, 3H, ArH), 5.28 (t, $J = 10.0$ Hz, 1H, H-3), 4.57 (d, $J = 10.0$ Hz, 1H, H-1), 3.49–3.44 (m, 2H, H-2 & H-5), 3.29 (t, $J = 10.0$ Hz, 1H, H-4), 1.45 (d, $J = 6.2$ Hz, 3H, CH_3).

^{13}C NMR (100 MHz, CDCl_3) δ 165.5, 133.9, 133.8, 130.9, 130.1, 129.3, 128.9, 128.8, 128.7, 86.3, 75.3, 75.1, 65.9, 63.6, 18.8.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{19}\text{H}_{18}\text{N}_6\text{O}_3\text{NaS}$, 433.1059; found, 433.1055.

11a. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 7.66–7.63 (m, 4H, ArH), 7.58–7.51 (m, 2H, ArH), 7.47–7.37 (m, 6H, ArH), 7.31–7.28 (m, 3H, ArH), 4.95 (dd, $J = 10.0, 3.6$ Hz, 1H, H-3), 4.39 (d, $J = 10.0$ Hz, 1H, H-1), 4.16 (d, $J = 3.6$ Hz, 1H, H-4), 3.84–3.70 (m, 2H, H-6a, 6b), 3.72–3.62 (m, 2H, H-2, H-5), 2.19 (s, 3H, CH_3), 1.05 (s, 9H, $(\text{CH}_3)_3\text{CSi}$).

^{13}C NMR (100 MHz, CDCl_3) δ 170.0, 135.7, 133.4, 132.94, 132.89, 131.2, 130.1, 129.2, 128.6, 128.0, 86.9, 77.2, 75.2, 62.3, 59.9, 59.6, 27.0, 20.8, 19.3.

11b. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.5

^1H NMR (400 MHz, CDCl_3) δ 8.10 (d, $J = 6.8$ Hz, 2H, ArH), 7.66–7.60 (m, 5H, ArH), 7.56–7.40 (m, 10H, ArH), 7.38–7.31 (m, 3H, ArH), 5.24 (dd, $J = 10.0, 3.5$ Hz, 1H, H-3), 4.52 (d, $J = 10.0$ Hz, 1H, H-1), 4.29 (d, $J = 3.5$ Hz, 1H, H-4), 3.90–3.69 (m, 3H, H-6a, 6b & H-5), 3.74 (t, $J = 10.0$ Hz, 1H, H-2), 1.05 (s, 9H, $(\text{CH}_3)_3\text{CSi}$).

^{13}C NMR (100 MHz, CDCl_3) δ 165.5, 135.6, 134.0, 133.3, 132.9, 132.8, 131.2, 130.2, 130.1, 129.2, 128.8, 128.6, 128.5, 128.0, 86.9, 77.3, 75.3, 62.3, 60.3, 59.9, 26.9, 19.3.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{35}\text{H}_{36}\text{N}_6\text{O}_4\text{NaSSi}$ 687.2186; found 687.2171.

12a. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.45

^1H NMR (400 MHz, CDCl_3) δ 7.76–7.74 (m, 2H, ArH), 7.69–7.66 (m, 2H, ArH), 7.64–7.61 (m, 2H, ArH), 7.46–7.37 (m, 6H, ArH), 7.30–7.24 (m, 3H, ArH), 4.77 (dd, $J = 10.0, 2.8$ Hz, 1H, H-3), 4.47 (d, $J = 10.0$ Hz, 1H, H-1), 4.28 (d, $J = 2.8$ Hz, 1H, H-4), 4.02 (dd, $J = 11.2, 4.0$ Hz, 1H, H-6a), 3.92 (dd, $J = 11.2, 4.0$ Hz, 1H, H-6b), 3.85 (t, $J = 10.0$ Hz, 1H, H-2), 3.61 (bs, 1H, OH), 3.51 (t, $J = 4.0$ Hz, 1H, H-5), 2.17 (s, 3H, CH_3), 1.07 (s, 9H, $(\text{CH}_3)_3\text{CSi}$).

^{13}C NMR (100 MHz, CDCl_3) δ 170.3, 135.8, 135.7, 133.6, 132.4, 132.0, 131.2, 130.27, 130.23, 129.24, 128.5, 128.1, 128.0, 86.5, 76.7, 75.8, 68.4, 65.2, 59.2, 26.8, 21.2, 19.2.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{30}\text{H}_{35}\text{N}_3\text{O}_5\text{NaSSi}$ 600.1964; found 600.1980.

12b. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.45

^1H NMR (400 MHz, CDCl_3) δ 8.08 (d, $J = 6.8$ Hz, 2H, ArH), 7.76–7.55 (m, 7H, ArH), 7.47–7.36 (m, 8H, ArH), 7.33–7.24 (m, 3H, ArH), 4.98 (dd, $J = 10.0$, 2.8 Hz, 1H, H-3), 4.55 (d, $J = 10.0$ Hz, 1H, H-1), 4.44 (d, $J = 2.8$ Hz, 1H, H-4), 4.05–3.93 (m, 3H, H-6a, 6b & H-2), 3.60 (t, $J = 4.0$ Hz, 1H, H-5), 3.33 (bs, 1H, OH), 1.06 (s, 9H, $(\text{CH}_3)_3\text{CSi}$).

^{13}C NMR (100 MHz, CDCl_3) δ 165.9, 135.8, 135.7, 133.7, 133.5, 132.5, 132.2, 131.5, 130.24, 130.21, 130.1, 129.4, 129.2, 128.6, 128.5, 128.12, 128.09, 86.8, 77.5, 76.6, 68.2, 64.9, 59.7, 26.9, 19.2.

HRMS calculated for $\text{C}_{35}\text{H}_{37}\text{O}_5\text{N}_3\text{Si}$ $[\text{M} + \text{Na}]^+$ 662.2121; found 662.2112.

14. TLC (petroleum ether:ethyl acetate, 9:1 vol/vol) R_f 0.4

^1H NMR (400 MHz, CDCl_3) δ 7.65–7.61 (m, 6H, ArH), 7.57–7.37 (m, 6H, ArH), 7.29–7.27 (m, 3H, ArH), 5.43 (d, $J = 3.2$ Hz, 1H, H-4), 4.46 (d, $J = 10.0$ Hz, 1H, H-1), 3.80–3.77 (m, 2H, H-3 & H-5), 3.75–3.68 (m, 2H, H-6a & H-6b), 3.48 (t, $J = 10.0$ Hz, 1H, H-2), 2.01 (s, 3H, CH_3), 1.04 (s, 9H, $(\text{CH}_3)_3\text{CSi}$).

^{13}C NMR (100 MHz, CDCl_3) δ 171.7, 135.7, 133.1, 133.0, 132.9, 131.9, 130.08, 130.04, 129.1, 128.3, 128.0, 127.97, 127.96, 86.8, 77.3, 73.5, 69.1, 62.6, 61.6, 26.9, 20.8, 19.2.

HR-ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{30}\text{H}_{35}\text{N}_3\text{O}_5\text{NaSSi}$, 600.1964; found, 600.1940.

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- Hitchen, P.G. & Dell, A. Bacterial glycoproteomics. *Microbiology* **152**, 1575–1580 (2006).
- Dube, D.H., Champasa, K. & Wang, B. Chemical tools to discover and target bacterial glycoproteins. *Chem. Commun.* **47**, 87–101 (2011).
- Stallforth, P., Lepenies, B., Adibekian, A. & Seeberger, P.H. Carbohydrates: a frontier in medicinal chemistry. *J. Med. Chem.* **52**, 5561–5577 (2009).
- Boltje, T.J., Buskas, T. & Boons, G.-J. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. *Nat. Chem.* **1**, 611–622 (2009).
- Morelli, L., Poletti, L. & Lay, L. Carbohydrates and immunology: synthetic oligosaccharide antigens for vaccine formulation. *Eur. J. Org. Chem.* 5723–5777 (2011).
- Stimson, E. *et al.* Meningococcal pilin: a glycoprotein substituted with digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose. *Mol. Microbiol.* **17**, 1201–1214 (1995).
- Young, N.M. *et al.* Structure of the N-linked glycan present on multiple glycoproteins in the gram-negative bacterium, *Campylobacter jejuni*. *J. Biol. Chem.* **277**, 42530–42539 (2002).
- Castric, P., Cassels, F.J. & Carlson, R.W. Structural characterization of the *Pseudomonas aeruginosa* 1244 pilin glycan. *J. Biol. Chem.* **276**, 26479–26485 (2001).
- Kenne, L., Lindberg, B., Petersson, K., Katzenellenbogen, E. & Romanowska, E. Structural studies of the O-specific side-chains of the *Shigella sonnei* phase I lipopolysaccharide. *Carbohydr. Res.* **78**, 119–126 (1980).
- Lindberg, B., Lindqvist, B., Lönngren, J. & Powell, D.A. Structural studies of the capsular polysaccharides from *Streptococcus pneumoniae* type 1. *Carbohydr. Res.* **78**, 111–117 (1980).
- Baumann, H., Tzianabos, A.O., Brissón, J.-R., Kasper, D.L. & Jennings, H.J. Structural elucidation of two capsular polysaccharides from one strain of *Bacteroides fragilis* using high-resolution NMR spectroscopy. *Biochemistry* **31**, 4081–4089 (1992).

- Bergström, N., Jansson, P.-E., Kilian, M. & Sørensen, U.B.S. Structures of two cell wall-associated polysaccharide of a *Streptococcus mitis* biovar 1 strain. *Eur. J. Biochem.* **267**, 7147–7157 (2000).
- Karlsson, C., Jansson, P.-E. & Sørensen, U.B.S. The pneumococcal common antigen C-polysaccharide occurs in different forms. *Eur. J. Biochem.* **265**, 1091–1097 (1999).
- Ovchinnikova, O.G. *et al.* Structure of the O-polysaccharide of *Providencia alcalifaciens* O22 containing D-glyceramide 2-phosphate. *Eur. J. Org. Chem.* **2012**, 3500–3506 (2012).
- Candela, T. *et al.* Environmental and biofilm-dependent changes in a *Bacillus cereus* secondary cell wall polysaccharide. *J. Biol. Chem.* **286**, 31250–31262 (2011).
- Filice, M., Guisan, J.M. & Palomo, J.M. Recent trends in regioselective protection and deprotection of monosaccharides. *Curr. Org. Chem.* **14**, 516–532 (2010).
- Filice, M. & Palomo, J.M. Monosaccharide derivatives as central scaffolds in the synthesis of glycosylated drugs. *RSC Adv.* **2**, 1729–1742 (2012).
- Lee, D. & Taylor, M.S. Catalyst-controlled regioselective reactions of carbohydrate derivatives. *Synthesis* **44**, 3421–3431 (2012).
- Filice, M., Guisan, J.M., Terreni, M. & Palomo, J.M. Regioselective monodeprotection of peracetylated carbohydrates. *Nat. Protoc.* **7**, 1783–1796 (2012).
- Pastore, A., Valerio, S., Adinolfi, M. & Iadonisi, A. An easy and versatile approach for the regioselective de-O-benzoylation of protected sugars based on the $\text{I}_2/\text{Et}_3\text{SiH}$ combined system. *Chem. Eur. J.* **17**, 5881–5889 (2011).
- Wang, C.-C. *et al.* Regioselective one-pot protection of carbohydrates. *Nature* **446**, 896–899 (2007).
- Wang, C.-C., Kulkarni, S.S., Lee, J.-C., Luo, S.-Y. & Hung, S.-C. Regioselective one-pot protection of glucose. *Nat. Protoc.* **3**, 97–113 (2008).
- Huang, T.Y., Zueletta, M.M.L. & Hung, S.-C. One-pot strategies for the synthesis of the tetrasaccharide linkage of proteoglycans. *Org. Lett.* **13**, 1506–1509 (2011).
- Chang, K.-L., Zulueta, M.M.L., Lu, X.-A., Zhong, Y.-Q. & Hung, S.-C. Regioselective one-pot protection of D-glucosamine. *J. Org. Chem.* **75**, 7424–7427 (2010).
- Patil, P.S., Lee, C.-C., Huang, Y.-W., Zulueta, M.M.L. & Hung, S.-C. Regioselective and stereoselective benzyldiene installation and one-pot protection of D-mannose. *Org. Biomol. Chem.* **11**, 2605–2612 (2013).
- Wang, C.-C., Zulueta, M.M.L. & Hung, S.-C. Regioselective one-pot protection and protection-glycosylation of carbohydrates. *Chimia* **65**, 54–58 (2011).
- François, A., Urban, D. & Beau, J.-M. Tandem catalysis for a one-pot regioselective protection of carbohydrates: the example of glucose. *Angew. Chem. Int. Ed.* **46**, 8662–8665 (2007).
- Muramatsu, W., Mishiro, K., Ueda, Y., Furuta, T. & Kawabata, T. Perfectly regioselective and sequential protection of glucopyranosides. *Eur. J. Org. Chem.* 827–831 (2010).
- Enugala, R., Carvalho, L.C.R. & Marques, M.M.B. Towards glucosamine building blocks: regioselective one-pot protection and deallylation procedures. *Synlett* 2711–2716 (2010).

30. Bourdreux, Y., Lemetais, A., Urban, D. & Beau, J.-M. Iron (III) chloride-tandem catalysis for a one-pot regioselective protection of glycopyranosides. *Chem. Commun.* **47**, 2146–2148 (2011).
31. Joseph, A.A. *et al.* TMSOTf-catalyzed silylation: streamlined regioselective one-pot protection and acetylation of carbohydrates. *Eur. J. Org. Chem.* 744–753 (2012).
32. Chang, S.-M., Tu, Z., Jan, H.-M., Pan, J.-F. & Lin, C.-H. Rapid synthesis of oligomannosides with orthogonally protected monosaccharides. *Chem. Commun.* **49**, 4265–4267 (2013).
33. Jones, G.B., Lin, Y., Xiao, Z., Kappen, L. & Goldberg, I.H. Molecular probes of DNA bulges: functional assay and spectroscopic analysis. *Bioorg. Med. Chem.* **15**, 784–790 (2007).
34. Amin, M.N., Ishiwata, A. & Ito, Y. Synthesis of asparagine-linked bacillosamine. *Carbohydr. Res.* **341**, 1922–1929 (2006).
35. Weerapana, E., Glover, K.J., Chen, M.M. & Imperiali, B. Investing bacterial N-linked glycosylation: synthesis and glycosyl acceptor activity of the undecaprenyl pyrophosphate-linked bacillosamine. *J. Am. Chem. Soc.* **127**, 13766–13767 (2005).
36. Liav, A., Hildesheim, J., Zehavi, U. & Sharon, N. Synthesis of 2-acetamido-2,6-dideoxy-D-glucose (*N*-acetyl-D-quinovosamine), 2-acetamido-2,6-dideoxy-D-galactose (*N*-acetyl-D-fucosamine), and 2,4-diacetamido-2,4,6-trideoxy-D-glucose from 2-acetamido-2-deoxy-D-glucose. *Carbohydr. Res.* **33**, 217–227 (1974).
37. Medgyes, A., Farkas, E., Lipták, A. & Pozsgay, V. Synthesis of the monosaccharide units of the O-specific polysaccharide of *Shigella sonnei*. *Tetrahedron* **53**, 4159–4178 (1997).
38. Smid, P. *et al.* Stereoselective synthesis of a dimer containing an α -linked 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (SUGp) unit. *J. Carbohydr. Chem.* **11**, 849–865 (1992).
39. Pedersen, C.M. *et al.* Total synthesis of lipoteichoic acid of *Streptococcus pneumoniae*. *Angew. Chem. Int. Ed.* **49**, 2585–2590 (2010).
40. Pragani, R., Stallforth, P. & Seeberger, P.H. *De novo* synthesis of a 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) building block for the preparation of a *Bacteroides fragilis* A1 polysaccharide fragment. *Org. Lett.* **12**, 1624–1627 (2010).
41. Schmölder, C., Nowikow, C., Kählig, H. & Schmid, W. Gram scale *de novo* synthesis of 2,4-diacetamido-2,4,6-trideoxy-D-galactose. *Carbohydr. Res.* **367**, 1–4 (2013).
42. Leonori, D. & Seeberger, P.H. *De novo* synthesis of the bacterial 2-amino-2,6-dideoxy sugar building blocks D-fucosamine, D-bacillosamine, and D-xylo-6-deoxy-4-ketohexosamine. *Org. Lett.* **14**, 4954–4957 (2012).
43. Emmadi, M. & Kulkarni, S.S. Rapid transformation of D-mannose into orthogonally protected D-glucosamine and D-galactosamine thioglycosides. *J. Org. Chem.* **76**, 4703–4709 (2011).
44. Emmadi, M. & Kulkarni, S.S. Expedient synthesis of bacterial, rare sugar building blocks to access the prokaryotic glycome. *Org. Biomol. Chem.* **11**, 3098–3102 (2013).
45. Emmadi, M. & Kulkarni, S.S. Orthogonally protected D-galactosamine thioglycoside building blocks via highly regioselective, double serial and double parallel inversions of β -D-thiomannoside. *Org. Biomol. Chem.* **11**, 4825–4830 (2013).
46. Pedretti, V., Veyrières, A. & Sinaÿ, P. A novel 1,3 O $\rightarrow$ C silyl rearrangement in carbohydrate chemistry: synthesis of α -D-glycopyranosyltrimethylsilanes. *Tetrahedron* **46**, 77–88 (1990).
47. Vos, J.N., van Boom, J.H., van Boeckel, C.A.A. & Beetz, T. Azide substitution at mannose derivatives. A potential new route to glucosamine derivatives. *J. Carbohydr. Chem.* **3**, 117–124 (1984).
48. Hederos, M. & Konradsson, P. Efficient routes to ethyl-2-deoxy-2-phthalimido-1- β -D-thio-galactosamine derivatives via epimerization of the corresponding glucosamine compounds. *J. Carbohydr. Chem.* **24**, 297–320 (2005).
49. Dong, H., Pei, Z., Angelin, M., Byström, S. & Ramström, O. Efficient synthesis of β -D-mannosides and β -D-talosides by double parallel or double serial inversion. *J. Org. Chem.* **72**, 3694–3701 (2007).
50. Ko, S.Y. *et al.* Total synthesis of the L-hexoses. *Science* **220**, 949–951 (1983).
51. Ko, S.Y. *et al.* Total synthesis of the L-hexoses. *Tetrahedron* **46**, 245–264 (1990).
52. Babu, R.S., Zhou, M. & O'Doherty, G.A. *De novo* synthesis of oligosaccharides using a palladium-catalyzed glycosylation reaction. *J. Am. Chem. Soc.* **126**, 3428–3429 (2004).
53. Shan, M. & O'Doherty, G.A. *De novo* asymmetric syntheses of SL0101 and its analogues via a palladium-catalyzed glycosylation. *Org. Lett.* **8**, 5149–5152 (2006).
54. Guo, H. & O'Doherty, G.A. *De novo* asymmetric synthesis of the anthrax tetrasaccharide by a palladium-catalyzed glycosylation reaction. *Angew. Chem. Int. Ed.* **46**, 5206–5208 (2007).
55. Wu, B., Li, M. & O'Doherty, G.A. Synthesis of several cleistrioside and cleistetroside natural products via a divergent *de novo* asymmetric approach. *Org. Lett.* **12**, 5466–5469 (2010).
56. Northrup, A.B. & Macmillan, D.W.C. Two-step synthesis of carbohydrates by selective aldol reactions. *Science* **305**, 1752–1755 (2004).
57. Demizu, Y. *et al.* Regioselective protection of sugars catalyzed by dimethyltin dichloride. *Org. Lett.* **10**, 5075–5077 (2008).
58. Lattrell, R. & Lohaus, G. Studies on the total synthesis of cephalosporin derivatives. II. – Substitution reactions with *trans*-3-sulfonyloxy-2-azetidinones. – A synthesis of *cis*-3-acylamino-4-alkylthio-2-azetidinones. *Liebigs Ann. Chem.* **1974**, 901–920 (1974).
59. Albert, R., Dax, K., Link, R.W. & Stütz, A.E. Carbohydrate triflates: reaction with nitrite, leading directly to epi-hydroxy compounds. *Carbohydr. Res.* **118**, C5–C6 (1983).
60. Dong, H. *et al.* Control of the ambident reactivity of the nitrite ion. *Org. Biomol. Chem.* **11**, 648–653 (2013).
61. Cai, Y., Ling, C.-C. & Bundle, D.R. Concise and efficient synthesis of 2-acetamido-2-deoxy- β -D-hexopyranosides of diverse aminosugars from 2-acetamido-2-deoxy- β -D-glucose. *J. Org. Chem.* **74**, 580–589 (2009).
62. Bongat, A.F.G. & Demchenko, A.V. Recent trends in the synthesis of O-glycosides of 2-amino-2-deoxysugars. *Carbohydr. Res.* **342**, 374–406 (2007).
63. Seeberger, P.H. Automated oligosaccharide synthesis. *Chem. Soc. Rev.* **37**, 19–28 (2008).
64. Liu, F., Aubry, A.J., Schoenhofen, I.C., Logan, S.M. & Tanner, M.E. The engineering of bacteria bearing azido-pseudaminic acid-modified flagella. *ChemBioChem* **10**, 1317–1320 (2009).
65. Swarts, B.M. *et al.* Probing the mycobacterial trehalose with bioorthogonal chemistry. *J. Am. Chem. Soc.* **134**, 16123–16126 (2012).
66. Backus, K.M. *et al.* Uptake of unnatural trehalose analogs as a reporter for *Mycobacterium tuberculosis*. *Nat. Chem. Biol.* **7**, 228–235 (2011).