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Expeditious synthesis of bacterial, rare sugar building blocks to access the prokaryotic glycome†

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Bacteria have unusual glycans which are not accessible by isolation. Herein, we describe a general and divergent strategy for the synthesis of the rare, bacterial deoxy amino hexopyranoside building blocks from D-mannose. The methodology is applied to the first total synthesis of the L-serine linked trisaccharide of *Neisseria meningitidis*.

Introduction

Protein glycosylation, which was once believed to be restricted to eukaryotes, is now shown to be common in prokaryotes.[1] A major difference between the two, however, lies in their glycan structures. The bacterial glycoproteins and oligosaccharides contain rare deoxy amino sugars which are not present on the human cell surface. This important structural difference which helps one to distinguish between the pathogen and the host cell could be exploited for target specific drug discovery and more importantly for carbohydrate based vaccine development.[2]

In contrast to their eukaryotic counterparts, bacterial glycoproteins have atypical monosaccharides attached to L-serine or L-threonine (Fig. 1). For example, *O*-linked glycans located on the pilin of *Neisseria gonorrhoeae* and *Neisseria meningitidis* consist of 2,4-diacetamido-2,4,6-trideoxyhexose (DATDH),[3] whereas *N*-linked glycans of *C. jejuni*[4] comprise a Bacillosamine (Bac) core. Likewise, *N*-acetyl fucosamine (FucNAc) is found in *O*-linked glycans on flagellin of *Pseudomonas aeruginosa*.[5] The 2-acetamido-4-amino-2,4,6-trideoxyhexose (AAT) forms a key component of the zwitterionic polysaccharides (ZPSs) located on the surfaces of various bacteria.[6–10] Vaccine candidates against these bacteria are highly desired. For this purpose, access to chemically pure and structurally well defined antigenic material is essential. However, the bacterial carbohydrates could not be isolated from natural sources in acceptable amounts and purity. Thus, chemical synthesis of orthogonally protected building blocks of the rare deoxy amino sugars is pivotal for synthesizing the bacterial glycans.

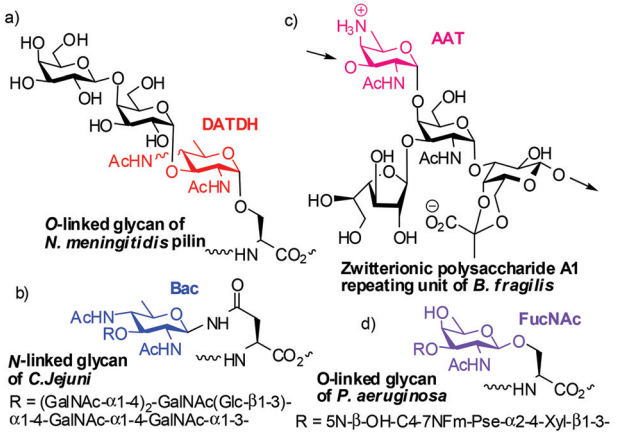

Fig. 1 Structures of various bacterial glycans containing rare deoxy amino sugars.

Given their biological importance, several routes are reported for the synthesis of the rare sugars. While many of these monosaccharide building blocks could be synthesized by classical carbohydrate approaches,[11–17] those strategies mostly involve lengthy routes with extensive protecting group manipulations and/or expensive starting materials. Very recently a *de novo* strategy has been developed for AAT and other related deoxy amino sugars from L-threonine[18,19] and L-Garner aldehyde,[20] respectively. These elegant routes also involve a number of steps and intermittent separation of diastereomers. A general and simple route for accessing all the rare sugar building blocks is still missing. We reasoned 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 building blocks, if the number of protection–deprotection steps could be decreased

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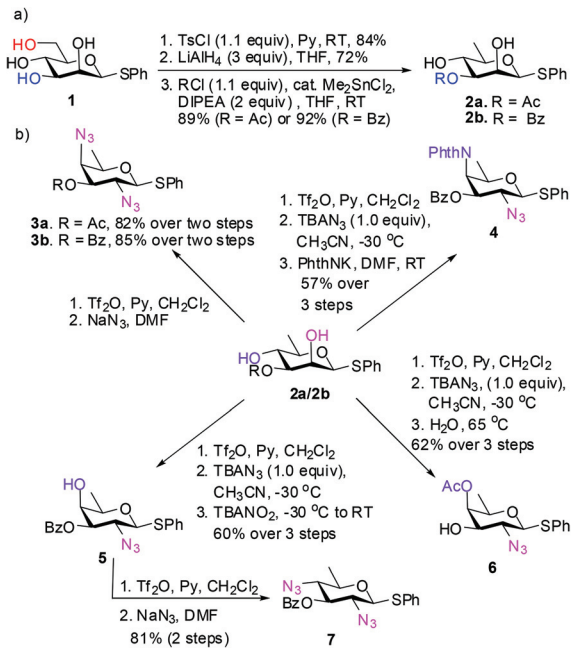
by carrying out regioselective and one-pot reactions. Here we present a short, efficient and general protocol for the synthesis of various orthogonally protected, bacterial rare sugar building blocks. Our strategy involves a one-pot double serial and double parallel displacement of the D-mannose derived 2,4-bis-trifluoromethanesulfonates (OTf, triflate) by azide, phthalimide and nitrite ions as nucleophiles. By changing the sequence of addition of nucleophiles, various orthogonally protected rare deoxy amino sugar building blocks could be rapidly synthesized. The inherent potential of the methodology is exemplified by the first total synthesis of the L-serine linked trisaccharide of *Neisseria meningitidis*.

Results and discussion

Previous works in this field have shown that the presence of the β-linkage in D-mannoside[21,22] and the 3-*O*-acyl group[22–24] are crucial for achieving success in nucleophilic displacements using azide or nitrite anions, respectively. As shown in Scheme 1, the primary hydroxyl group of **1**[25] was regioselectively tosylated and subsequently reduced with LAH to form the corresponding C6-deoxy sugar (D-rhamnose), which upon a highly regioselective tin mediated acylation[26] with AcCl or BzCl afforded 2,4-diols **2a** and **2b**, respectively, which served as common intermediates to fashion various rare sugar building blocks. Triflation of **2a** and **2b**, followed by the S_N2 displacement of the formed bis-triflates by a brief treatment with excess NaN_3, cleanly generated the 2,4-diazido derivatives (DATDH) **3a** (82%) and **3b** (85%), respectively, with the inversion of stereochemistry at C2 and C4, in an essentially one-pot manner.



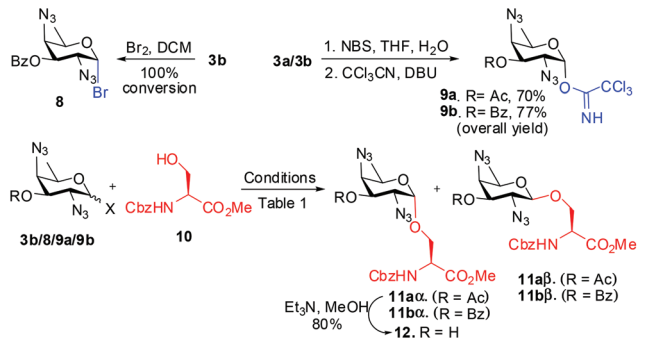
Scheme 1 Synthesis of bacterial, rare deoxy amino monosaccharide building blocks.

A sequential double serial C2, C4 inversion *via* displacement of triflates was attempted next. Compound **2b** was first treated with Tf_2O to obtain the corresponding 2,4-bis-triflate, which upon treatment with a stoichiometric amount of tetrabutylammonium azide ($TBAN_3$) in CH_3CN at −30 °C smoothly underwent a highly regioselective displacement of the C-2 triflyloxy group, exclusively. Evaporation of the solvent and addition of potassium phthalimide (2 equiv.) in DMF in the same pot at RT displaced the remaining C4-OTf and afforded the AAT building block **4** in 57% overall yield. The regioselectivity in this case is mainly governed by steric factors. Apparently, the C2-OTf of the β-isomers **2a**/**2b** is more accessible as compared to the C4-OTf. It is further tuned by employing an exact stoichiometric amount of the reagent and conducting the displacement at a low temperature.

The high regioselectivity observed in the above reaction prompted us to try a Lattrell–Dax reaction[27–30] involving nucleophilic displacement of triflate using nitrite anions. Thus, compound **2b** was subjected to triflation and concomitant treatment with $TBAN_3$ (1.0 equiv.) in CH_3CN under similar conditions at −30 °C. After the completion of the reaction as indicated by TLC, tetrabutylammonium nitrite ($TBANO_2$) was added in the same pot, to displace the 4-OTf and generate the D-fucosamine derivative **5**, exclusively, in 60% overall yield. In order to access the D-fucosamine derivative with a 3-OH free, a different approach was used. Starting with the C3-acetate **2a**, the first two steps were repeated in an identical manner to obtain the corresponding 2-azido-4-OTf (quinovosamine) intermediate, as judged by TLC. Then, water was added and the reaction mixture was heated to 65 °C to generate 3-hydroxy fucosamine **6** as a sole product in 62% overall yield. Here, the 3-*O*-acetyl group migrated from C-3 to C-4 along the top face to displace the C4-triflyloxy group with inversion at C-4. The Bac building block **7** was synthesized from **5** by sequential triflation followed by azide displacement with C4 inversion in an essentially one-pot manner (81%). In this way, most of the bacterial rare sugar building blocks **3**–**7** could be procured in an expedient manner from a common intermediate **2a** or **2b** *via* regioselective tandem reactions, after a single chromatographic purification.‡ These valuable thioglycoside building blocks can be utilized as stable and flexible glycosyl donors or acceptors to stereoselectively assemble the bacterial glycoproteins and oligosaccharides.

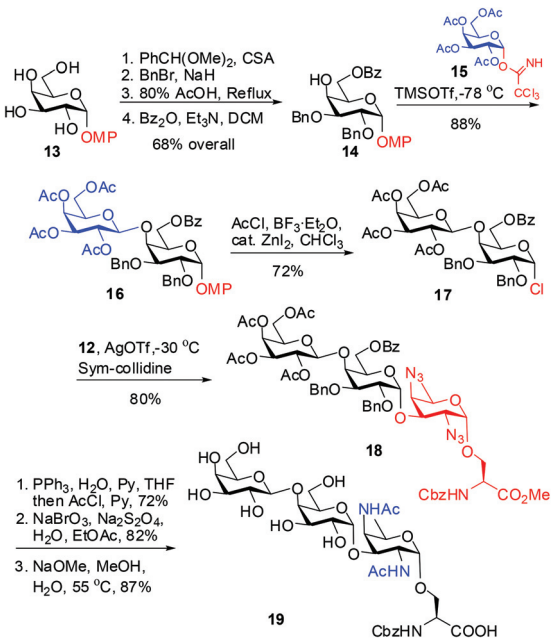
As an application of our methodology, we report herein the first total synthesis of the α-L-serine linked trisaccharide of *N. meningitidis* (Scheme 2). The *O*-linked trisaccharide was identified in *N. meningitidis* pili, the filamentous glycoproteins that are essential adhesins in capsular bacteria.[3] Its structure was proposed based on the linkage analysis by acid hydrolysis and mass spectroscopic studies and has been shown to comprise the DATDH sugar attached to L-serine; the stereochemistry at C4 of the DATDH sugar was not defined (Fig. 1a). A major challenge in the synthesis was the stereoselective installation of two consecutive α-linkages in **19**. Achieving α-stereoselectivity in coupling of the 6-deoxy monosaccharide with the primary OH of the amino acid L-serine was very

Table 1 Stereoselective glycosylation of L-serine **10** with various donors



Entry	Donor	Promoter	Temp. (°C)	Solvent	α/β	Yield (%)
1	**3b**	Ph_2SO/Tf_2O	−60	CH_2Cl_2	2.2/1	78
2	**8**	AgOTf	−50	CH_2Cl_2	1.8/1	65
3	**8**	$AgClO_4$	RT	CH_2Cl_2	2.0/1	69
4	**8**	TBAI	RT	CH_2Cl_2	1.0/0	29
5	**9a/9b**	TMSOTf	−78	THF	1.0/0	92/93

difficult. Advantageously, all our building blocks, being stable thioglycosides, could be readily transformed into other types of donors (Table 1). Of the various glycosyl donors tried, the trichloroacetimidates worked the best (Table 1, entry 5). Coupling of donor **9a/9b** with L-serine acceptor **10** using TMSOTf as a promoter at −78 °C in THF as a participating solvent cleanly generated a single α-isomer **11aα/11bα** individually, in 92% and 93% yield, respectively. Selective removal of an acetate group in **11aα** using Et_3N and methanol afforded acceptor **12** (80%). In parallel, the α-methoxyphenyl digalactoside **16** (Scheme 2) was prepared from a known OMP galactoside **13** in 5 steps *via* glycosylation of 4-OH acceptor **14** with imidate **15**.‡



Scheme 2 Synthesis of the α-L-serine linked trisaccharide of *N. meningitidis*.

It has been reported that the α-anomeric OMP glycosides are reluctant to react with AcCl, $ZnCl_2$ and cat. ZnI_2 to form glycosyl chlorides whereas the β-isomers react readily.[31] However, we found that, by using a combination of AcCl, $BF_3{\cdot}OEt_2$ and cat. ZnI_2, the α-anomeric OMP in **16** could be easily replaced by chloride and the so formed α-glycosyl chloride **17** was coupled with the glycosyl amino acid acceptor **12** using AgOTf as a promoter to afford exclusively α-linked product **18** (^{1}H NMR δ 4.74, d, $J_{1',2'}$ = 3.6 Hz, H-1′)‡ in 80% yield. The α-selectivity in this case is presumably arising through the intermediacy of the β-triflate. Global deprotection of **18** involving Staudinger reduction of azides, *N*-acetylation, oxidative debenzylation,[32] and de-*O*-acetylation afforded the target molecule **19** in 51% overall yield.

Conclusions

In conclusion, we have developed a short and efficient protocol to synthesize most of the bacterial rare deoxy amino hexoses (FucNAc, Bac, AAT, and DATDH) as orthogonally protected, stable thioglycoside building blocks. Thus, D-mannose can be expediently transformed into various bacterial aminosugar thioglycosides in a few steps. The methodology is applied to the first synthesis of the L-serine linked trisaccharide of *N. meningitidis*. The short and efficient protocol is expected to speed up bacterial glycan assembly and give rapid access to the prokaryotic glycome.

Experimental

All reactions were conducted under a dry nitrogen atmosphere. Solvents (CH_2Cl_2 >99%, THF 99.5%, acetonitrile 99.8%, DMF 99.5%) were purchased in capped bottles and dried under sodium or CaH_2. All other solvents and reagents were used without further purification. All glassware was oven-dried before use. TLC was performed on precoated aluminum plates of silica gel 60 F254 (0.25 mm, E. Merck). Developed TLC plates were visualized under a short-wave UV lamp and by heating plates that were dipped in ammonium molybdate/cerium(IV) sulfate solution. Silica gel column chromatography was performed using silica gel (100–200 mesh) and employed a solvent polarity correlated with TLC mobility. NMR experiments were conducted on a Bruker 400 MHz instrument using $CDCl_3$ (D, 99.8%) or $(CD_3)_2CO$ (D, 99.9% or CD_3OD 99.8%) as solvents. Chemical shifts are relative to the deuterated solvent peaks and are in parts per million (ppm). ^{1}H–^{1}H COSY and HSQC were used to confirm proton assignments. Mass spectra were acquired in the ESI mode.

Phenyl 2-azido-3-*O*-benzoyl-2,4,6-trideoxy-4-phthalimido-1-thio-β-D-galactopyranoside (4)

Trifluoromethanesulfonic anhydride (0.42 mL, 2.5 mmol) was added dropwise at −10 °C to a stirred solution of **2b** (0.15 g, 0.4 mmol), pyridine (0.43 mL, 5.4 mmol) in CH_2Cl_2 (7 mL)

and the solution was gradually brought to 10 °C over 2 h. After complete conversion of the starting material the reaction mixture was diluted with CH_2Cl_2 and washed successively with 1 M HCl, aq. $NaHCO_3$ and water. Combined organic layers were dried over Na_2SO_4, concentrated and the crude product was used for the next step without any purification.

The crude product was dissolved in acetonitrile (11 mL), and to this, $TBAN_3$ (118 mg, 0.42 mmol) was added at −30 °C and the reaction mixture was stirred at the same temperature for 20 h. After 20 h the solvent was evaporated on a rotary evaporator under an N_2 atmosphere and the residue was dissolved in DMF (2 mL). To this clear solution, PhthNK (0.15 g, 0.83 mmol) was added. After 10 h the reaction mixture was diluted with EtOAc and washed with water. The separated aqueous layer was washed with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (1 : 9 ethyl acetate–toluene) to obtain **4** as a viscous liquid (0.12 g, 57%). ^{1}H 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*): $[M + Na]^+$ calcd for $C_{27}H_{22}N_4O_5NaS$, 537.1209; found, 537.1134.

Phenyl 2-azido-3-*O*-benzoyl-2,6-dideoxy-1-thio-β-D-galactopyranoside (5)

Trifluoromethanesulfonic anhydride (1.0 mL, 6.0 mmol) and pyridine (1.0 mL, 13.0 mmol) were added sequentially at −10 °C to a stirred solution of **2b** (0.36 g, 1.0 mmol) in CH_2Cl_2 (15 mL). Then the reaction mixture was gradually warmed to 10 °C over 2 h. After complete consumption of the starting material, the reaction mixture was diluted with CH_2Cl_2 and washed successively with 1 M HCl, aq. $NaHCO_3$ and brine. The separated organic layer was dried over Na_2SO_4 and concentrated.

The crude product which was obtained after the removal of solvents was dissolved in acetonitrile (20 mL), and to this, $TBAN_3$ (0.28 g, 1.0 mmol) was added at −30 °C and the reaction mixture was stirred at the same temperature for 20 h. $TBANO_2$ (0.8 g, 3.0 mmol) was added and the reaction mixture was stirred at RT for 1 h. It was diluted with EtOAc and washed with water. The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (1 : 9 ethyl acetate–pet ether) to obtain **5** as a pale yellowish viscous liquid (0.23 g, 60%). ^{1}H 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*): $[M + Na]^+$ calcd for $C_{19}H_{19}N_3O_4NaS$, 408.0994; found, 408.0974.

Phenyl 4-*O*-acetyl-2-azido-2,6-dideoxy-1-thio-β-D-galactopyranoside (6)

Trifluoromethanesulfonic anhydride (0.83 mL, 4.9 mmol) and pyridine (0.86 mL, 10.7 mmol) were added sequentially at −10 °C to a stirred solution of **2a** (0.24 g, 0.82 mmol) in CH_2Cl_2 (12 mL). Then the reaction mixture was gradually warmed to 10 °C over 2 h. After complete consumption of the starting material, the reaction mixture was diluted with CH_2Cl_2 and washed successively with 1 M HCl, aq. $NaHCO_3$ and brine. The separated organic layer was dried over Na_2SO_4 and concentrated.

The crude product which was obtained after the removal of solvents was dissolved in acetonitrile (17 mL), and to this, $TBAN_3$ (0.23 g, 0.82 mmol) was added at −30 °C and the reaction mixture was stirred at the same temperature for 20 h. Then the reaction mixture was concentrated to 5 mL, and to this, water (0.33 mL, 18.0 mmol) was added and the reaction mixture was kept for reflux at 65 °C for 1 h. It was diluted with EtOAc and washed with water. The separated organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (1 : 9 ethyl acetate–pet ether) to obtain **6** as a pale yellowish viscous liquid (0.16 g, 62%). ^{1}H 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 and 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*): $[M + Na]^+$ calcd for $C_{14}H_{17}N_3O_4NaS$, 346.0837; found, 346.0819.

Acknowledgements

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‡See ESI.

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