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Expedient Route To Access Rare Deoxy Amino L-Sugar Building Blocks for the Assembly of Bacterial Glycoconjugates

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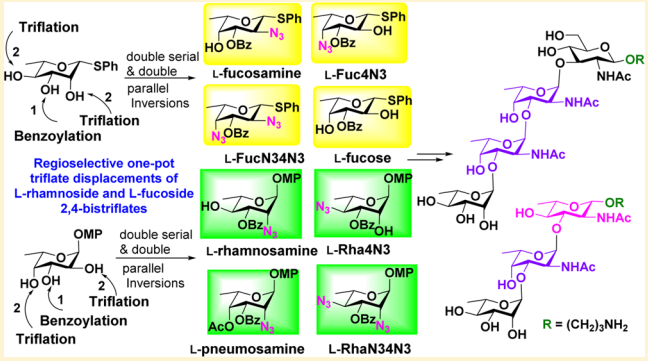
Supporting Information

ABSTRACT: Bacterial glycoproteins and oligosaccharides contain several rare deoxy amino L-sugars which are virtually absent in the human cells. This structural difference between the bacterial and host cell surface glycans can be exploited for the development of carbohydrate based vaccines and target specific drugs. However, the unusual deoxy amino L-sugars present in the bacterial glycoconjugates are not available from natural sources. Thus, procurement of orthogonally protected rare L-sugar building blocks through efficient chemical synthesis is a crucial step toward the synthesis of structurally well-defined and homogeneous complex glycans. Herein, we report a general and expedient methodology to access a variety of unusual deoxy amino L-sugars starting from readily available L-rhamnose and L-fucose via highly regioselective, one-pot double serial and double parallel displacements of the corresponding 2,4-bistriflates using azide and nitrite anions as nucleophiles. Alternatively, regioselective monotriflation at O2, O3, and O4 of L-rhamnose/L-fucose allowed selective inversions at respective positions leading to diverse rare sugars. The orthogonally protected deoxy amino L-sugar building blocks could be stereoselectively assembled to obtain biologically relevant bacterial *O*-glycans, as exemplified by the first total synthesis of the amino linker-attached, conjugation-ready tetrasaccharide of O-PS of *Yersinia enterocolitica* O:50 strain 3229 and the trisaccharide of *Pseudomonas chlororaphis* subsp. *aureofaciens* strain M71.



INTRODUCTION

Bacteria have unique glycan structures on their surface which differ from their eukaryotic counterparts.[1] This difference between the bacterial and human cell surfaces can be exploited for target specific drug discovery and vaccine development.[2] Unfortunately, the bacterial glycoproteins cannot be isolated from natural sources in pure form and acceptable amounts. Chemical synthesis of the complex bacterial glycoconjugates is therefore a crucial step toward realizing this goal.

Bacterial glycoconjugates are composed of several atypical amino 6-deoxy-L-sugars.[3] Some representative structures are shown in Figure 1. For example, 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl L-fucosamine, L-FucNAc) is present in *Yersinia enterocolitica* serotype O:50 strain 3229,[4] *Staphylococcus aureus*,[5] *Pseudomonas chlororaphis*,[6] *Plesiomonas shigelloides* serotope O1,[7] and *Pseudomonas aeruginosa* serotype O12.[8] Likewise, 2-acetamido-2,6-dideoxy-L-talose (*N*-acetyl 6-deoxy-L-talosamine), commonly known as *N*-acetyl L-pneumosamine (L-PneNAc), is a constituent of *P. shigelloides* serotope O1[7] and *Alteromonas nigrifaciens* IAM 13010^T.[9] The 2-acetamido-2,6-dideoxy-L-glucose (*N*-acetyl L-quinovosamine, L-QuiNAc) is present in *Vibrio vulnificus* BO62316,[10] *P. chlororaphis*,[6] *Proteus vulgaris* TG 155,[11] *V. vulnificus* MO6-24,[12] and *Shewanella putrefaciens* strain S29.[13] The 2-acetamido-2,6-dideoxy-L-rhamnose (*N*-acetyl L-rhamnosamine, L-RhaNAc) forms a key component of the surface glycans of *V. vulnificus* BO62316[10] and *P. vulgaris* TG 155 from a new *Proteus* serogroup O55.[11] These glycans being virtually absent on the human cell surfaces are expected to induce specific immune response in human hosts and are thus regarded as potential vaccine candidates against a variety of infectious diseases. On the other hand, derivatives of 4-amino-4,6-dideoxy L-sugars are present in potent antibiotics such as tallysomycin and kansosamine.[14] The 2,4-diamino-2,4,6-trideoxy-hexoses have also attracted attention due to their direct involvement in microbial pathogenecity.[15] Moreover, the 3-amino-3,6-dideoxy-L-talose and 3-amino-3,6-dideoxy-L-mannose (L-mycosamine) are components of structurally related antiviral antibiotics fluvirucins A1, A2, B1, B2, B3, B4, and B5, which are active against influenza A virus.[16]

Given their biological importance, these glycoconjugates are attractive synthetic targets. However, the rare deoxy amino L-sugars are not available from natural sources. Thus, procurement of the suitably protected rare deoxy amino L-sugar building blocks through chemical synthesis is a major impediment limiting the biological evaluation of the complex bacterial cell surface glycans. Although there are efficient methods reported in literature for the syntheses of deoxy amino D-sugars,[17] attempts toward the syntheses of their L-counterparts are rare. Several methods are available for the synthesis of

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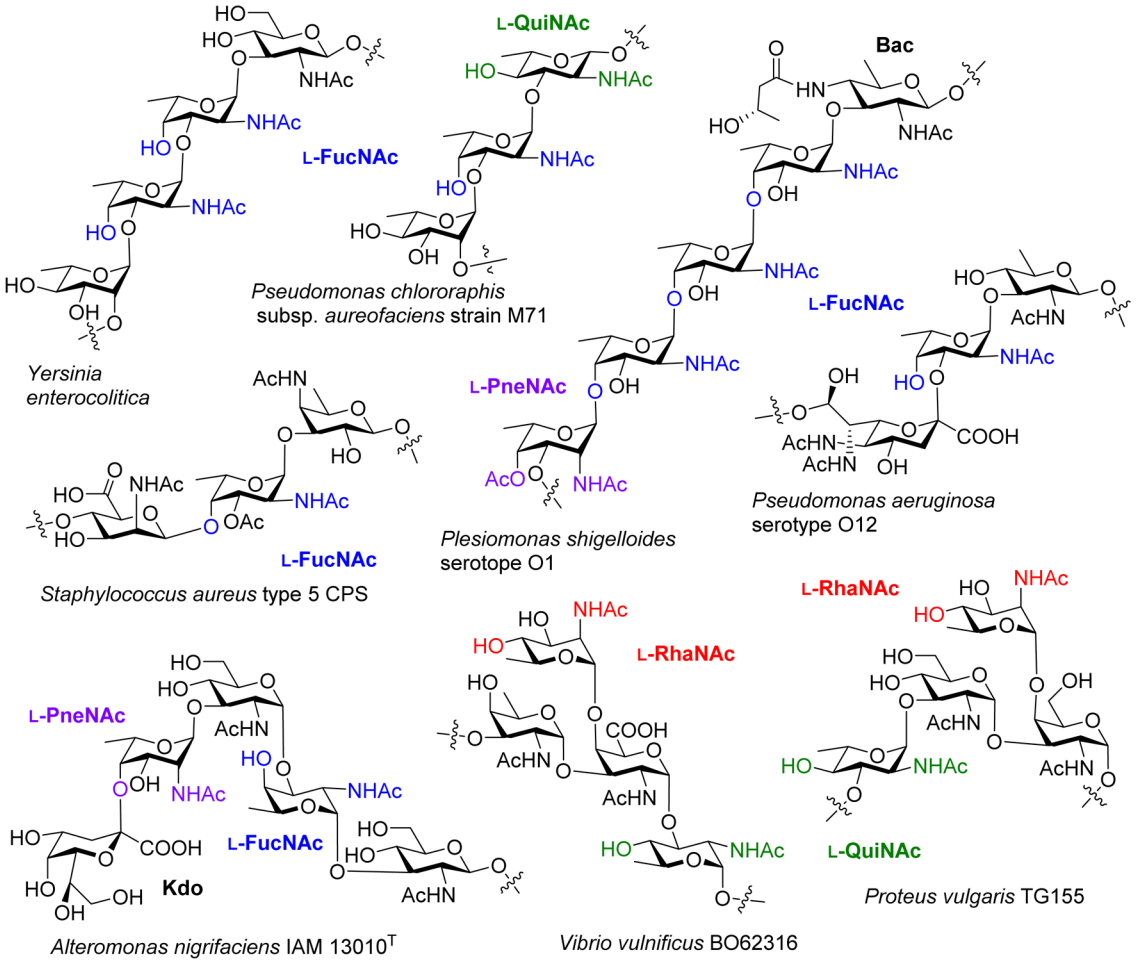

Figure 1. Representative structures of bacterial glycans containing rare deoxy amino L-sugars.

L-hexoses and 6-deoxy L-hexoses.[18] However, only a few reports are there on chemical synthesis of L-fucosamine,[19] L-quinovosamine,[20] L-rhamnosamine,[20,21] L-pneumosamine,[22] and 4-amino-4,6-dideoxy-L-sugars,[23] as well as 2,4-diamino-2,4,6-trideoxy-L-sugars[24] and 3-amino-3,6-dideoxy-L-sugars,[25] which mostly involve lengthy routes starting from carbohydrate precursors leading to low yields of products or diastereomeric mixtures. For this reason, the *de novo* approaches have been looked upon as viable alternatives in recent years. O'Doherty and co-workers employed their *de novo* methodology to construct 4-amino-4,6-dideoxy-L-rhamnose moiety of methymycin analogues starting from furan via postglycosylational transformations.[26] Very recently, Seeberger and co-workers extended their elegant *de novo* approach to the synthesis of L-FucNAc starting from D-Garner aldehyde.[27] Still, there is no general and divergent protocol to access differentially protected rare deoxy amino L-sugar building blocks that can be used as glycosyl donors or acceptors in the assembly of complex bacterial glycans. Therefore, we planned to develop a general and expedient methodology to synthesize a variety of rare amino deoxy L-sugars as thioglycosides or *p*-methoxyphenyl glycoside building blocks starting from the readily available L-rhamnose and L-fucose.

Our approach involved one-pot double serial or double parallel displacements of 2,4-bis-trifluoromethanesulfonates (OTf, triflate) of L-rhamnoside and L-fucoside by azide and/or nitrite anions as nucleophiles. We have recently established an efficient protocol to access rare deoxy amino D-sugars via the displacement of 2,4-bistriflates derived from D-mannose.[28,17] It was envisioned that such nucleophilic displacements on L-rhamnose or L-fucose scaffolds would provide a facile entry to diverse rare amino deoxy L-sugars. In conjunction with this, regioselective monotriflations at O2, O3, and O4 of L-rhamnose/L-fucose were expected to allow selective inversions at respective positions leading to a variety of rare sugars.

RESULTS AND DISCUSSION

Synthesis of Rare Deoxy Amino L-Sugar Building Blocks. Readily available *β*-L-thiorhamnoside **1**[29] was selected as a suitable starting material for our initial studies (Scheme 1a). A highly regioselective monobenzoylation of **1** using 5.0 mol % dimethyltin dichloride (Me_2SnCl_2),[30] benzoyl chloride (1.2 equiv), and DIPEA in THF cleanly generated the 3-OBz derivative **2** (95%). For the synthesis of L-quinovosamine, the 2,4-diol **2** was treated with *tert*-butyldimethyl silyl chloride (TBSCl) in the presence of imidazole to obtain 4-OTBS derivative **3** in 66% yield along with ~10% of the corresponding 2-OTBS derivative. The remaining 2-OH in **3** was converted to *O*-triflate, which was subsequently displaced by sodium azide (NaN_3) in DMF to give orthogonally protected 2-azido-2,6-dideoxy-L-glucose **4** (L-quinovosamine) in 78% yield over 2 steps, in a one-pot manner.

The 2,4-diol **2** served as a common precursor for accessing various rare sugar derivatives of L-fucosamine and L-fucose via

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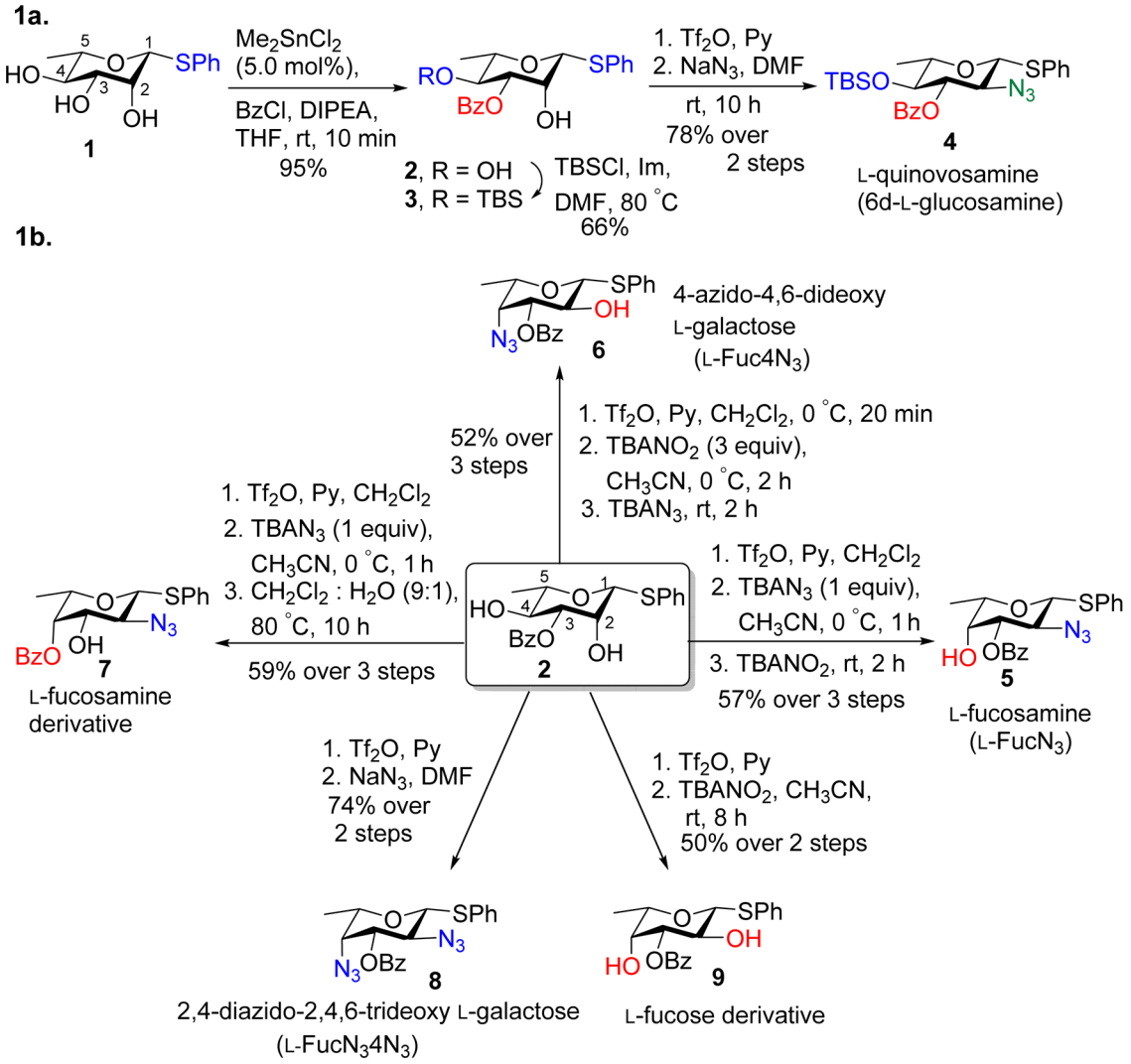
Scheme 1. Synthesis of L-Quinovosamine, L-Fucosamine, L-Fucose, L-Fuc4N$_3$, and L-FucN$_3$4N$_3$ Derivatives via Double Parallel and Double Serial Inversions



double serial and double parallel inversions of the corresponding L-rhamnosyl 2,4-bis-triflates using azide, and/or nitrite anions as nucleophiles (Scheme 1b). Throughout the studies, we carried out a brief aqueous workup after triflation to obtain a crude triflate derivative which was used as such in the subsequent steps. Column chromatography was performed only once at the end of each sequence of displacements. For the synthesis of L-fucosamine derivative **5**, compound **2** was treated with triflic anhydride (Tf_2O) in pyridine to afford the corresponding 2,4-bis-triflate, which, upon treatment with a stoichiometric amount of tetrabutyl ammonium azide ($TBAN_3$) in acetonitrile at 0 °C for 1 h, underwent a facile, regioselective displacement of the C2-OTf. Subsequent addition of 3 equiv of tetrabutyl ammonium nitrite ($TBANO_2$) in the same pot displaced the remaining C4-OTf, via a Lattrel–Dax reaction,[31] to afford 4-OH L-fucosamine derivative **5** in 57% yield over 3 steps. The double serial inversion also worked well upon reversing the order of the addition of nucleophiles. Accordingly, the 4-azido-4,6-dideoxy-L-galactose (L-Fuc4N$_3$) **6** was obtained via a highly regioselective displacement of C2-OTf with 3 equiv of $TBANO_2$ in acetonitrile at 0 °C for 2 h, and concomitant displacement of the C4-OTf by using 3 equiv of $TBAN_3$ in 52% yield over 3 steps. For the synthesis of 3-OH derivative of L-fucosamine **7**, compound **2** was converted to the corresponding 2,4-bistriflate, which was treated with 1 equiv of $TBAN_3$ in acetonitrile for 1 h at 0 °C followed by heating at 80 °C in a 9:1 CH_2Cl_2/H_2O solvent mixture to afford **7** (59% over 3 steps). In this water mediated transformation, the 3-OBz group displaces the C4-OTf from the bottom face to form a transient orthoester which concomitantly opens up selectively under the conditions to give axial 4-OBz group.[32] The reaction worked very well on a gram scale. Finally, the double parallel inversion of the 2,4-bistriflates with excess of sodium azide in dimethylformamide afforded 2,4-diazido-2,4,6-trideoxy-L-galactose derivative **8** in 74% yield over 2 steps. Similarly, treatment of the 2,4-bistriflates with 4 equiv of $TBANO_2$ in acetonitrile furnished L-fucose derivative **9** in 50% yield over 2 steps. In this way, we were able to rapidly transform L-rhamnose into various differentially protected derivatives of L-fucosamine and L-fucose in an efficient manner by involving one-pot transformations.

It should be however noted that the displacement of 2/4-*O*-triflates or 2,4-bistriflates of 3-*O*-benzoyl thio-α-L-rhamnoside and *p*-methoxyphenyl-α-L-rhamnosides did not work well and led to either decomposition or elimination products. These results are in congruence with the Richardson–Hogue rules[33]

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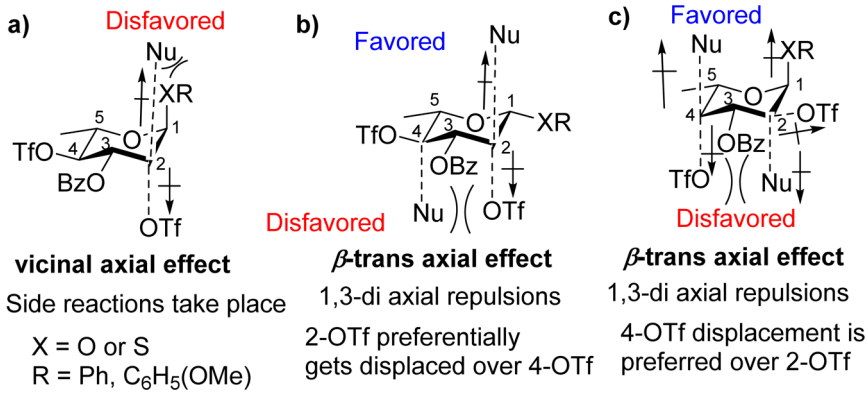
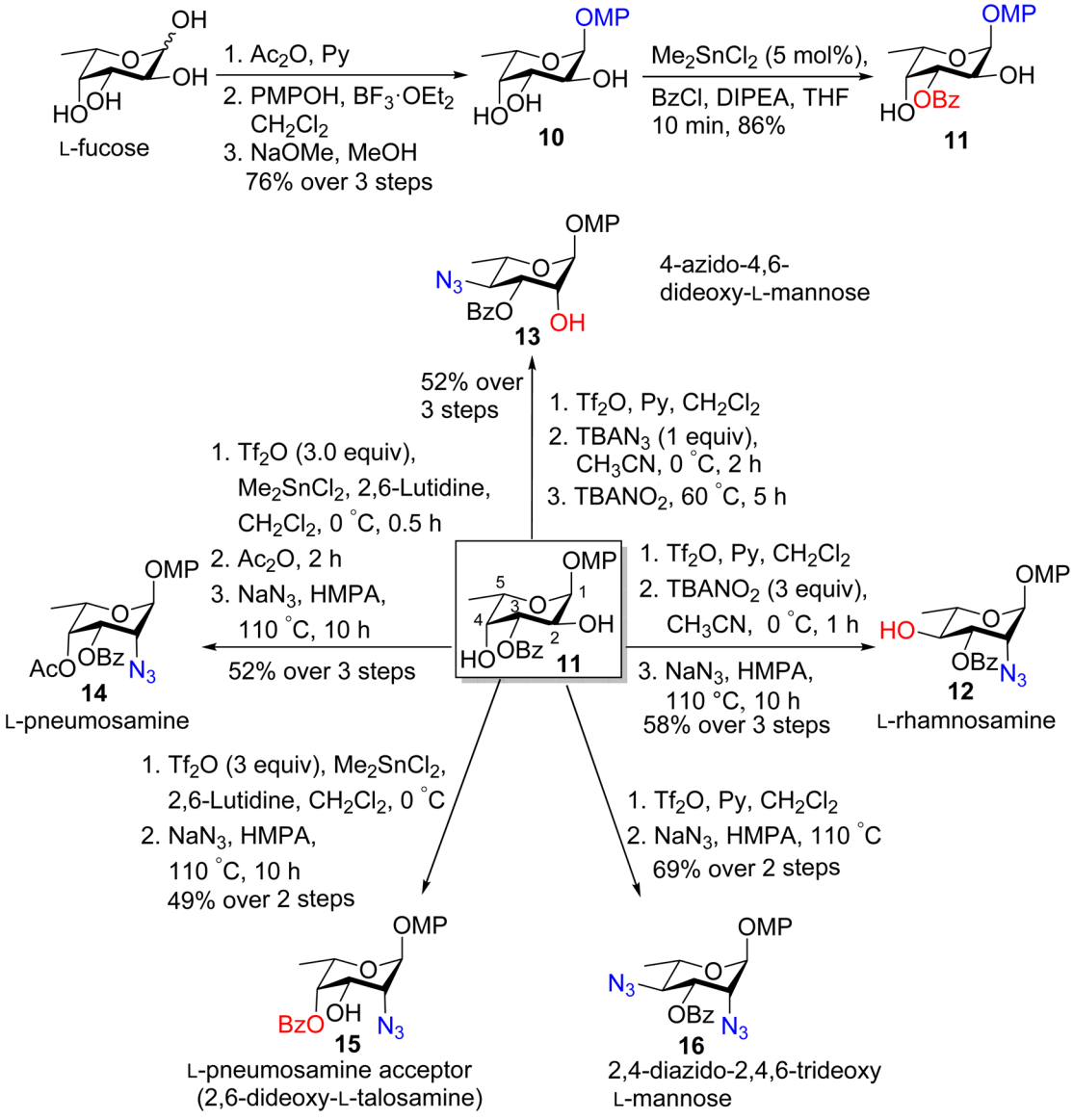


Figure 2. Explanation for the observed regioselectivity.

Scheme 2. Synthesis of L-Rhamnosamine, L-Pneumosamine, 4-Azido-L-mannoside, and 2,4-Diazido-L-mannoside via Double Parallel and Double Serial Inversions



for nucleophilic displacement of pyranoside triflates which are recently updated by Hale and co-workers.[34]

In the case of a α-L-rhamnoside, there is a strong unfavorable interaction between the anomeric *p*-methoxyphenyl group (or thiophenyl group) and the approaching nucleophile (Pyranoside Vicinal Axial Effect[34]) (Figure 2a) in the S_N2 transition state which leads to either E_2 elimination[35] (in the case of OMP) or decomposition (for thioglycoside through partic-

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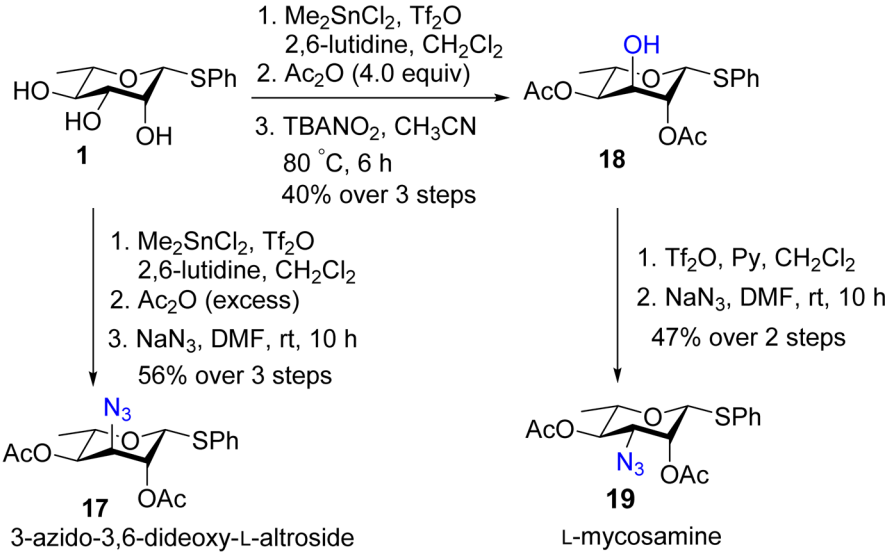
Scheme 3. Synthesis of 3-Azido-3,6-dideoxy-L-altroside 17 and L-Mycosamine 19



ipation of sulfur[29,36]). A mere change in the anomeric configuration from α to β alleviated such unfavorable repulsions facilitating successful displacement reactions (Figure 2b).

The regioselectivity attained in the triflate displacement reactions can be attributed to the differences in the steric crowding and stereoelectronic factors (β-trans axial effect[33,34] and 1,3-diaxial repulsions) at C2-OTf and C4-OTf of L-rhamnoside (Figure 2b). The equatorial C4-OTf on the β-L-rhamnoside scaffold (Figure 2b) is less accessible due to the presence of the axial C2-OTf which imparts a severe 1,3-diaxial repulsion as well as steric repulsion on the approaching nucleophile for a bottom face approach. Moreover, for the equatorial triflates to react, the pyranoses would have to undergo a ring flip, and therefore, much higher temperatures are required to achieve these transformations. In comparison, the axial C2-OTf is freely accessible for the nucleophile from the top face of the β-configured L-rhamnoside. Strategically, as soon as the axial C2-OTf is displaced, the C4-OTf becomes freely accessible for the incoming nucleophile. This setup may potentially lead to a double serial displacement of 2,4-bistriflates, which could be arrested at 0 °C using stoichiometric amount of $TBAN_3$ or controlled amount of $TBANO_2$. The C-4OTf could then be displaced concomitantly by another nucleophile (azide, nitrite, OBz) in a tandem one-pot manner. By extending the same logic, it was envisioned that, on the α-L-fucoside scaffold (Figure 2c), the axial C4-OTf would preferentially get displaced over the equatorial C2-OTf. In this case, the axial C4-OTf is expected to hinder the approaching nucleophile from the bottom face owing to steric and electronic repulsions. We anticipated that a very similar relative arrangement of substituents should allow us to carry out one-pot, regioselective displacements of L-fucosyl 2,4-bistriflates.

With these considerations, we began experimenting with L-fucose. The *p*-methoxyphenyl-α-L-fucoside **10** was first prepared from L-fucose following the reported procedure.[37] Accordingly, per-O-acetylation of L-fucose followed by nucleophilic displacement of the anomeric acetate with *p*-methoxyphenol using $BF_3{\cdot}Et_2O$ in CH_2Cl_2 and subsequent deacetylation provided triol **10** (Scheme 2). Regioselective 3-O-benzoylation of 2,3,4-triol **10** was achieved by using 5 mol % Me_2SnCl_2 and benzoyl chloride to afford **11** in 86% yield. The L-fucosyl 2,4-diol **11** was then treated with Tf_2O and pyridine to form the corresponding 2,4-bistriflate, which upon a brief workup was as such subjected to double serial and double parallel inversions to access the rare deoxy amino L-rhamno and L-talo derivatives. As anticipated, the 2,4-bistriflate, upon treatment with 3 equiv of $TBANO_2$ in acetonitrile at 0 °C for 1 h, underwent a highly regioselective displacement of the more accessible C4-OTf group; subsequent addition of NaN_3 in HMPA at 110 °C displaced the C2-OTf to afford L-rhamnosamine derivative **12** in 58% yield over 3 steps, after a single column chromatographic purification. Likewise, addition of a stoichiometric amount of $TBAN_3$ to the so formed 2,4-bistriflate in acetonitrile led to azide displacement of C4-OTf. Subsequent addition of $TBANO_2$ in the same pot and heating at 60 °C for 5 h generated 4-azido-2,6-dideoxy-L-mannose derivative **13** (52%, 3 steps). To synthesize 2-azido-2,6-dideoxy-L-talose (L-pneumosamine), we examined the catalytic Me_2SnCl_2 mediated regioselective 2-O-triflation of the 2,4-diol, capitalizing on the higher reactivity of the equatorial hydroxyl group and strong coordination ability of the 1,2-cis oriented oxygens with tin. Indeed, the 2,4-diol **11** upon treatment with 3 equiv of triflic anhydride in the presence of catalytic Me_2SnCl_2 and 2,6-lutidine in CH_2Cl_2 generated the corresponding 2-OTf derivative, exclusively (as judged by ^1H NMR). Sequential addition of acetic anhydride, to mask the remaining 4-OH, in the same pot and displacement of the C2-OTf by NaN_3 in HMPA as a solvent afforded the differentially protected L-pneumosamine derivative **14** in 52% yield over 3 steps. On the other hand, a regioselective C2-OTf formation of diol **11**, followed by its nucleophilic displacement with NaN_3 in HMPA at 110 °C for 10 h led to the formation of the 3-OH derivative of L-pneumosamine **15** in 49% over 2 steps, via a migration of the benzoyl group from 3-OH to 4-OH under the prevailing conditions. Thus, differentially protected L-pneumosamine derivatives could be obtained simply by capping the 4-OH by acetylation or by leaving it free to participate in the reaction. The double parallel displacement of the 2,4-bistriflate of L-fucoside **11** with sodium azide in HMPA at 110 °C generated the 2,4-diazido-2,4,6-trideoxy-L-mannoside **16** in 69% yield over 2 steps, uneventfully. In this way, we were able to rapidly access

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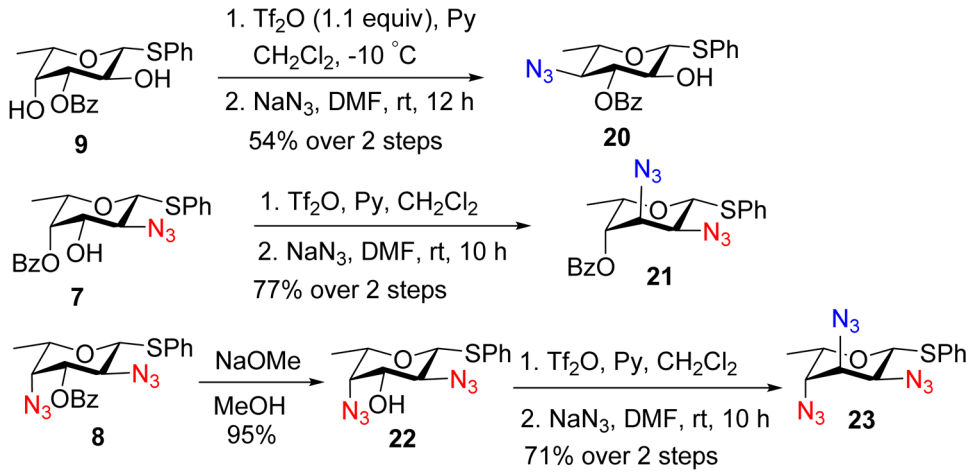
Scheme 4. Synthesis of 4-Azido-4,6-dideoxy-L-glucose 20, 2,3-Diazido 2,3,6-Trideoxy-L-guloside 21, and 2,3,4-Triazido-2,3,4,6-tetradeoxy-L-guloside 23



various differentially protected derivatives of L-rhamnosamine and L-pneumosamine from L-fucoside **11**.

To access the C3-functionalized rare L-sugars, we resorted to a regioselective triflation of the 2,3,4-triol. Synthesis of 3-azido-3,6-dideoxy-L-altroside was achieved via inversion of 3-OH of L-rhamnoside **1** by using regioselective 3-O-triflation and its sequential displacement (Scheme 3). Thus, regioselective 3-O-triflation using 3.5 equiv of Tf_2O, catalytic Me_2SnCl_2, and 2,6-lutidine in CH_2Cl_2 followed by acetylation of the 2,4-OH groups by addition of acetic anhydride in the same pot afforded the corresponding 3-*O*-triflyl-2,4-di-*O*-acetyl-L-rhamnoside derivative, which upon subsequent displacement of the C3-OTf by NaN_3 offered 3-azido-3,6-dideoxy-L-altroside **17** in 56% yield over 3 steps. Likewise, a regioselective 3-O-triflation of L-rhamnoside **1**, followed by acetylation of 2,4-hydroxyl groups and subsequent displacement of C3-OTf by $TBANO_2$ gave 6-deoxy-L-altroside **18** (40% yield over 3 steps). Triflation of 3-OH of **18** and concomitant displacement of the so formed C3-OTf with NaN_3 in HMPA fashioned L-mycosamine derivative **19** (47% yield over 2 steps).

In the course of our studies directed toward the synthesis of L-pneumosamine derivative, we observed an unexpected regioselectivity in triflation of L-thiofucoside **9** (Scheme 4). When compound **9** was treated with 1.1 equiv of Tf_2O and pyridine, we obtained the corresponding C4-OTf, exclusively, which could be concomitantly displaced with NaN_3 in the same pot to furnish 4-azido-L-glucose derivative **20** in 54% yield over 2 steps. The reason behind the observed unusual selectivity (4-OH axial over 2-OH equatorial) in triflation of 2,4-diol **9** could be attributed to the steric and electronic effects. Due to the equatorial disposition of the anomeric SPh group, as well as C3-OBz group, perhaps there is a steric hindrance for a bulky group such as a triflate to approach the C2-OH, in comparison with the freely accessible C4-OH. In addition to this, the SPh group being not a powerful electron withdrawing group does not cause appreciable difference in the acidity of C4-OH and C2-OH protons. Synthesis of 2,3-diazido-2,3,6-trideoxy-L-guloside **21** was achieved by inversion of 3-OH of L-fucosamine derivative **7**, via 3-O-triflation followed by displacement with NaN_3 in DMF in 77% yield over 2 steps. The rare sugar 2,3,4-triazido-2,3,4,6-tetradeoxy-L-guloside **23** was obtained from diazido compound **8**. Its debenzoylation gave 2-OH derivative **22**, which upon triflation and subsequent displacement by NaN_3 offered **23** in 71% yield over 2 steps in a one-pot manner. It should be noted that direct displacement of the corresponding L-rhamnosyl 2,3,4-tristriflate, derived from triol **1**, with NaN_3 failed to give **23** and led to the elimination of the axial C2-OTf instead.

In this way, an expedient protocol has been established for the synthesis of differentially protected phenylthio or *p*-methoxyphenyl glycosides of rare amino deoxy-L-sugars from readily available L-rhamnose or L-fucose via one-pot tandem nucleophilic displacements of *O*-triflates. We have also optimized reaction conditions for one-pot double serial and double parallel inversions of L-rhamnosyl 2,4-bistriflates to access L-fucosamine, L-fucose, L-Fuc4N_3, and L-Fuc$N_3$4N_3 derivatives in good overall yields. An azide displacement of orthogonally protected L-rhamnosyl C2-OTf afforded L-quinovosamine derivative. Similarly, L-rhamnosamine, 4-azido-4,6-dideoxy-L-mannoside, and 2,4-diazido-2,4,6-trideoxy-L-mannosides were obtained from L-fucoside. Alternatively, regioselective monotriflation at O2, O3, and O4 of L-rhamnose/L-fucose allowed facile entry to L-pneumosamine (6-deoxy-L-talosamine), L-mycosamine, and other rare sugars through inversion of respective positions. All the rare sugar building blocks synthesized in this study are either thioglycosides or methoxyphenyl glycosides which can be employed in glycosylation reactions as stable donors and acceptors. Ready availability of the rare deoxy amino L-sugar building blocks will expedite the synthesis of complex, rare sugar containing bacterial glycans, thereby allowing us to study their role in pathogenesis and their immunological properties for further development of vaccines.

Application to Total Synthesis of Bacterial *O*-Glycans. As an application of our methodology, we report herein the first total synthesis of the amino linker-attached, conjugation-ready tetrasaccharide of O-PS of *Y. enterocolitica* O:50 strain 3229 (Figure 3, **24**) and the trisaccharide of *P. chlororaphis* subsp. *aureofaciens* strain M71 (Figure 3, **25**), respectively. O-specific polysaccharide (O-PS) biological repeating unit of *Y. enterocolitica* serotype O:50 strain 3229 was isolated in 2012[4] and the structure was elucidated as →2)-*α*-L-Rha*p*-(1→3)-*α*-L-Fuc*p*NAc-(1→3)-*α*-L-Fuc*p*NAc-(1→3)-*β*-D-Glc*p*NAc-(1. *Y. enterocolitica* is clinically important among 17 Gram-negative species of *Yersinia* genus.[38] *Y. enterocolitica* most often causes enterocolitis, acute diarrhea, mesenteric lymphadentis, and

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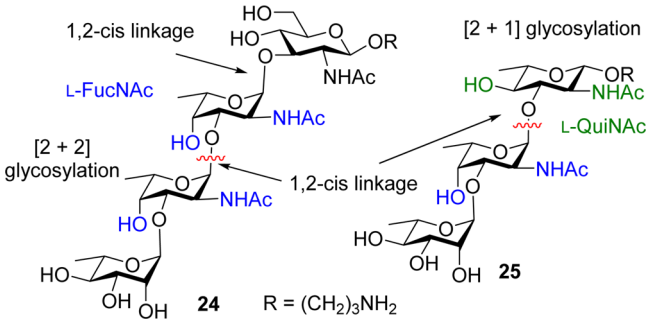
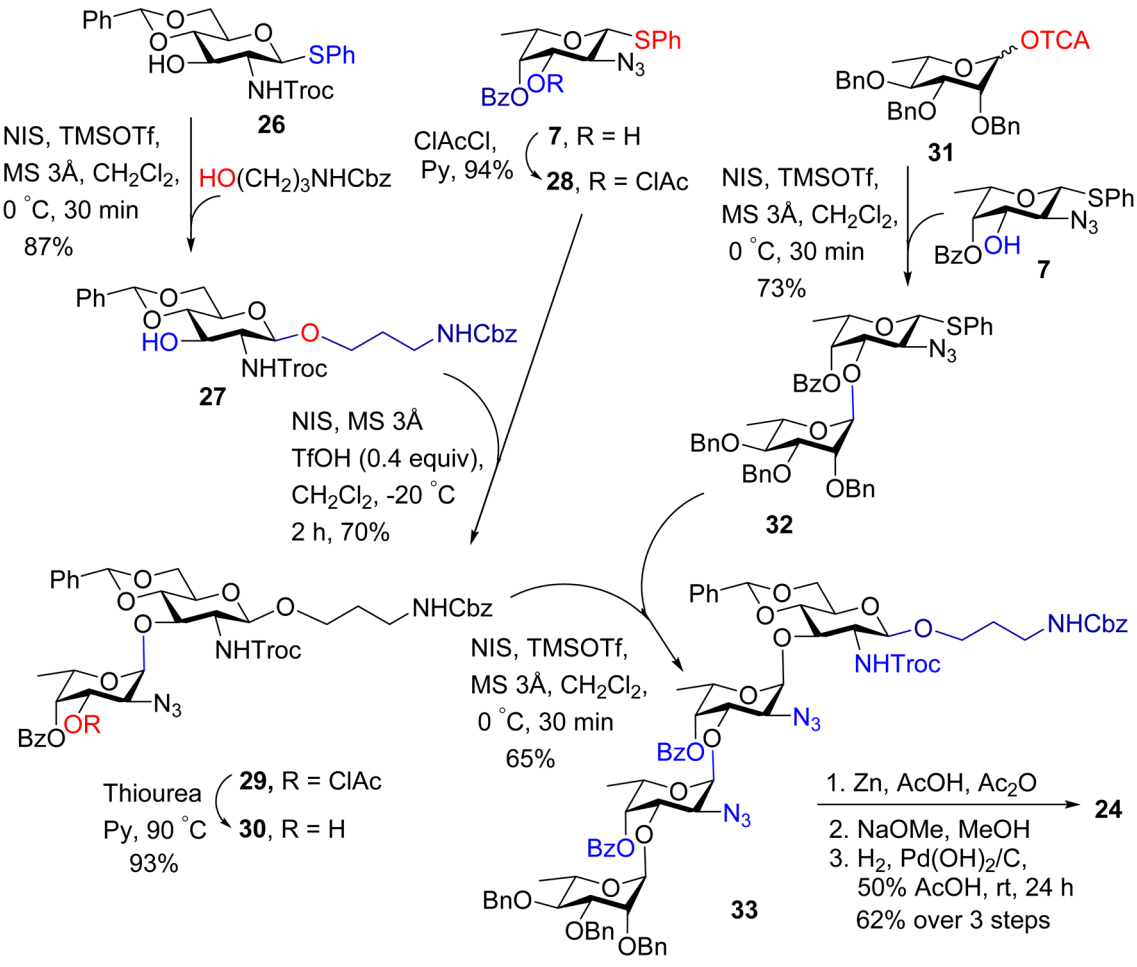


Figure 3. Structures of tetrasaccharide and trisaccharide repeating units of O-PS of *Y. enterocolitica* and *P. chlororaphis* subsp. *aureofaciens* strain M71, respectively.

pseudoappendicitis.[39] A structurally related O-specific trisaccharide →2)-α-L-Rha*p*-(1→3)-α-L-Fuc*p*NAc-(1→3)-β-L-Qui*p*NAc-(1→ was isolated from the root of a tomato plant by the mild acid hydrolysis of the lipopolysaccharide from *P. chlororaphis* subsp. *aureofaciens* strain M71.[6] This compound was able to inhibit the in vitro growth of *Seiridium cardinale* and other cypress pathogenic fungi as *Diplodia cupressi*, *Seridium cupressi*, and *Seridium unicorne*.[40]

The major challenges in the synthesis of the tetrasaccharide **24** are synthesis of appropriately protected L-fucosamine building block and installation of consecutive 1,2-cis linkages. For a convergent synthesis of tetrasaccharide **24**, we envisaged that a [2 + 2] glycosylation would be a better option (Figure 3). This would entail formation of two α-linked disaccharides, both containing the rare L-fucosamine unit, and their α-stereoselective assembly. Advantageously, the same nonreducing end disaccharide could be utilized for the synthesis of the trisaccharide **25** by coupling with appropriately protected L-quinovosamine derivative. As shown in Scheme 5, we began with the synthesis of the reducing end disaccharide **29**. A regioselective coupling between known **26**[41] and the amino linker $HO(CH_2)_3NHCbz$ using NIS and TMSOTf as activator in CH_2Cl_2 furnished the β-linked product **27** in 87% yield (β-linkage, δ 4.39, J = 7.5 Hz, $^1J_{C,H}$ = 158.8 Hz). The 3-OH of **7** was capped using chloroacetyl chloride and pyridine to afford the fully protected β-thio-L-fucoside donor **28** (94% yield), which was subsequently glycosylated with the 3-OH D-glucosamine acceptor **27** under NIS and TfOH promotion in CH_2Cl_2 to afford the desired α-linked disaccharide **29** in 70% yield (α-linkage, δ 4.90, J = 2.4 Hz, $^1J_{C,H}$ = 172.5). The observed exclusive α-selectivity can be attributed to the stabilization of the glycosyl cation intermediate through the anchimeric assistance of the strategically positioned 4-*O*-ester group.[42] Removal of the chloro acetyl group by treating **29** with thiourea gave 3′-OH **30** (93%), a suitable acceptor for the key [2 + 2] glycosylation. To synthesize the nonreducing end disaccharide **32**, trichloroacetimidate donor **31**[43] and acceptor **7** were coupled in the presence of TMSOTf to afford the α-linked disaccharide **32** in 73% yield (α-linkage, δ 5.21, J = 1.6

Scheme 5. Synthesis of Tetrasaccharide 24



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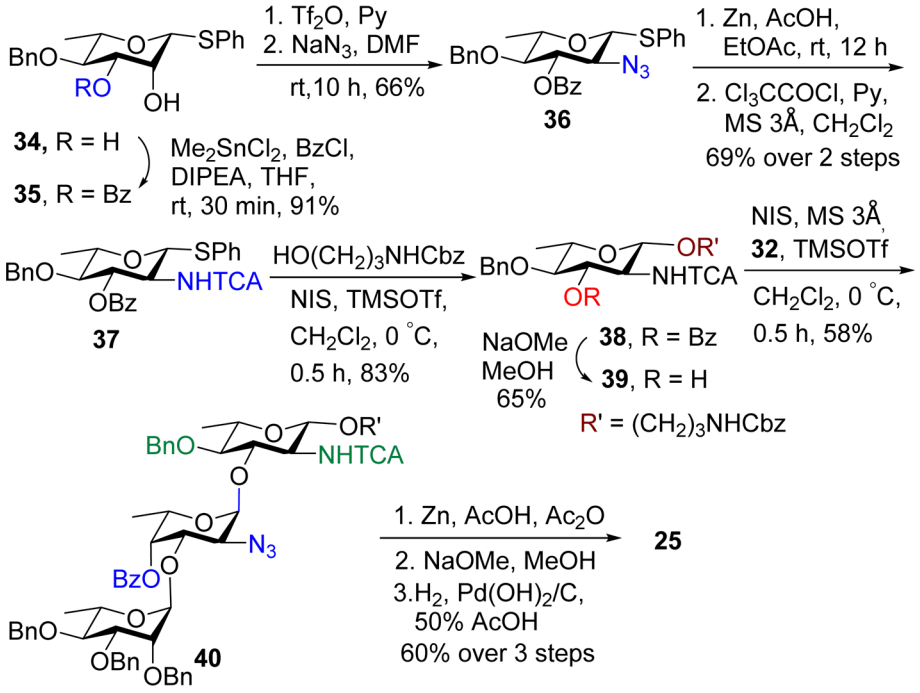
Scheme 6. Synthesis of Trisaccharide 25



Hz, $^{1}J_{C,H}$ = 167.6 Hz). The crucial coupling between disaccharide donor **32** and the reducing end disaccharide acceptor **30** in the presence of NIS and TMSOTf in CH_2Cl_2 afforded tetrasaccharide **33** in 65% yield (α-linkage, δ 5.36, J = 4.0 Hz, $^{1}J_{C,H}$ = 172.5 Hz). The ^{13}C NMR spectrum displayed peaks at 98.8 ($^{1}J_{C,H}$ = 175.0 Hz), 94.4 ($^{1}J_{C,H}$ = 168.7 Hz), 94.1 ppm ($^{1}J_{C,H}$ = 172.5 Hz) for α and 100.8 ppm ($^{1}J_{C,H}$ = 162.0 Hz) for β-anomeric carbons, respectively.

Global deprotection of tetrasaccharide **33** was achieved in 3 steps. Conversion of the azide and NHTroc to the corresponding acetamido group in a one-pot conversion was achieved by treatment with Zn/AcOH and Ac_2O. Debenzoylation with 2 N NaOMe in methanol followed by debenzylation and benzylidene deprotection was carried out under hydrogenation conditions using $H_2/Pd(OH)_2$ in 50% acetic acid to afford the target tetrasaccharide **24** in 62% over 3 steps, after purification over Sephadex G25 column. In this way, we have successfully completed the first total synthesis of a conjugation ready tetrasaccharide **24** of the O-PS from *Y. enterocolitica*. The installation of consecutive 1,2-cis linkages of L-fucosamine residues were achieved by exploiting the neighboring group participation of 4-OBz group.

The disaccharide **32** was also utilized in the assembly of trisaccharide **25** belonging to *P. chlororaphis* subsp. *aureofaciens* strain M71, as shown in Scheme 6. Diol **34** could be easily prepared from triol **1** following the procedure reported by Crich and co-workers.[29] Catalytic Me_2SnCl_2 mediated regioselective 3-O-benzoylation of 2,3-diol **34** provided 2-OH derivative **35** in 91% yield. The remaining 2-OH was triflated and displaced with azide to furnish L-quinovosamine derivative **36** (66% over 2 steps). Reduction of azide by using zinc in acetic acid and ethyl acetate gave the corresponding amine, which was capped as a trichloroacetate to obtain **37** (69% over 2 steps). Glycosylation of thioglycoside donor **37** with $OH(CH_2)_3NHCbz$ linker as an acceptor in the presence of NIS and TMSOTf afforded **38** in 83% yield (β-linkage, δ 4.41, J = 8.5 Hz, $^{1}J_{C,H}$ = 160.0 Hz). Debenzoylation of **38** using NaOMe in methanol provided **39** (65%), which was employed as an acceptor in the ensuing glycosylation. Finally, the disaccharide donor **32** was coupled with L-quinovosamine acceptor **39** in the presence of NIS and TMSOTf in CH_2Cl_2 at 0 °C to furnish α-linked trisaccharide **40** in 65% yield (α-linkage, δ 5.61, J = 4.0 Hz, $^{1}J_{C,H}$ = 171.5 Hz). Global deprotection of trisaccharide **40** was accomplished in 3 steps, in a similar manner. Conversion of azide and NHTCA to the corresponding acetamido group in a one-pot conversion (Zn/AcOH and Ac_2O) followed by debenzoylation using 2 N NaOMe in methanol and subsequent debenzylation under hydrogenation conditions using $H_2/Pd(OH)_2$ in 50% acetic acid smoothly delivered the target trisaccharide **25** in 60% yield over 3 steps.

CONCLUSION

In conclusion, we have established an expedient and facile protocol to synthesize differentially protected rare deoxy amino L-sugars of bacterial origin from readily available L-rhamnose and L-fucose in good overall yields. We employed one-pot double serial and double parallel inversions of *O*-triflates or regioslective triflation as powerful method to access most of the rare deoxy amino L-sugars present in bacteria. We extended this methodology to the total synthesis of O-PS repeating units of *Y. enterocolitica* and *P. chlororaphis* subsp. *aureofaciens* strain M71. The methodology will expedite assembly of bacterial glycoconjugates and speed up the vaccine development. The azide bearing rare sugars can be also utilized for metabolic incorporation of glycans to discover new bacterial glycoproteins and for target specific drugs.[44] The rare L-sugar building blocks will also serve as valuable tools to delineate the biosynthetic pathways of various infectious bacteria. This would further open up avenues for the development of novel antibiotics.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b01823.

Experimental procedures, characterization data for all new compounds, and copies of ^{1}H, ^{13}C and 2D NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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