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Article

Total Synthesis of the Repeating Units of Highly Functionalized *O*-Antigens of *Pseudomonas aeruginosa* ATCC 27577, O10, and O19

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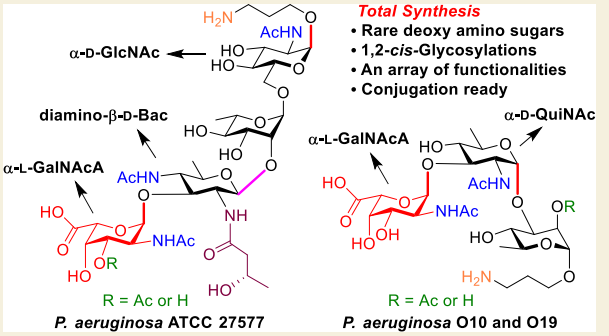
ABSTRACT: The first total synthesis of the repeating units of the *O*-antigens of *Pseudomonas aeruginosa* ATCC 27577, O10, and O19 was achieved via a linear glycosylation strategy. This also represents the first synthesis of an oligosaccharide containing an α-linked *N*-acetyl-L-galactosaminuronic acid (L-Gal*p*NAcA) unit. All of the glycosyl linkages, including three challenging 1,2-*cis*-glycosidic bonds of amino sugars, were effectively constructed with high to exclusive stereoselectivity, while orthogonal protection tactics were employed to facilitate regioselective glycosylations and the introduction of a variety of functionalities. An acetyl group migration phenomenon was found during the synthesis of the *O*-acylated repeating unit of the *P. aeruginosa* ATCC 27577 antigen. All synthetic targets carried an amino functional group in the linker at the reducing end, thus facilitating further regioselective elaboration and biological studies. The synthetic strategy established here should be useful for the preparation of other similar oligosaccharides.



KEYWORDS: *bacterial oligosaccharides, rare amino sugars, stereoselective glycosylation, Pseudomonas aeruginosa, α-linked L-GalpNAcA*

INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium and is ubiquitously present in diverse natural environments.[1,2] As an opportunistic human pathogen, it causes a range of acute and chronic diseases, such as cystic fibrosis and hospital-acquired pneumonia, as well as the infections in bloodstream, urinary tract, and burn wounds.[3−5] Due to the high levels of intrinsic and acquired antibiotic resistance, the U.S. Centers for Disease Control and Prevention has designated it as one of the "ESKAPE" pathogens resulting in severe nosocomial infections worldwide.[6,7] Besides, the World Health Organization has ranked *P. aeruginosa* as a critical priority for the development of new therapeutic strategies.[8] However, to date, no vaccines against *P. aeruginosa* have been licensed.

The lipopolysaccharides (LPSs) of *P. aeruginosa*, one of the major virulent factors, are exposed on the cell surface and play a vital role in the interaction of the bacterium and host immune system.[9,10] As the outer components of LPSs, their repetitive *O*-polysaccharide antigens contain a number of rare sugars, such as monoamino and diamino sugars, and some of them carry a carboxyl group. These sugars are not present in human cell glycans, making them excellent targets for the development of carbohydrate-based vaccines.[11,12] According to their structurally unique *O*-antigens, *P. aeruginosa* can be classified into 20 serotypes and more than 30 subtypes.[9,13−15] In the context of the structural heterogeneity of natural glycans from bacteria, synthetic oligosaccharides with well-defined structures and contaminant-free properties have been receiving growing attention.[16−19] In addition to being excellent haptens for developing carbohydrate-based antibacterial vaccines, the synthetic oligosaccharides are useful chemical biology tools for detailed studies and structure−activity relationship analyses of bacterial glycans as immunogens.[20−23]

The acidic LPS *O*-antigen of *P. aeruginosa* ATCC 27577 (IID 1001) (Figure 1), which was elucidated by Ito and co-workers,[24] is composed of a repeating tetrasaccharide: 4-α-L-Gal*p*NAcA-(1 → 3)-β-D-Bac*p*2N[(*S*)-3-hydroxybutyrylamido)]4NAc-(1 → 2)-α-L-Rha*p*-(1 → 6)-α-D-Glc*p*NAc-(1 →. About 80% of the repeating tetrasaccharides contain an acetyl group at the L-GalNAcA 3-*O*-position, and similar nonstoichiometric *O*-acetylation is very common in the LPS *O*-polysaccharides of *P. aeruginosa*.[9] This tetrasaccharide shows high structural similarity with the repeating units of the LPS *O*-antigens of *P. aeruginosa* Habs O3, Wokatsch O13, and O14, with only slight difference in the *N*/*O*-acylation pattern and the regiochemistry of the glycosidic linkage between the D-Bac and L-Rha units.[25] Besides, the nonreducing end disaccharide motif, α-L-GalNAcA-(1 → 3)-β-D-Bac, and its derivatives also appear in many other bacterial glycans, such as

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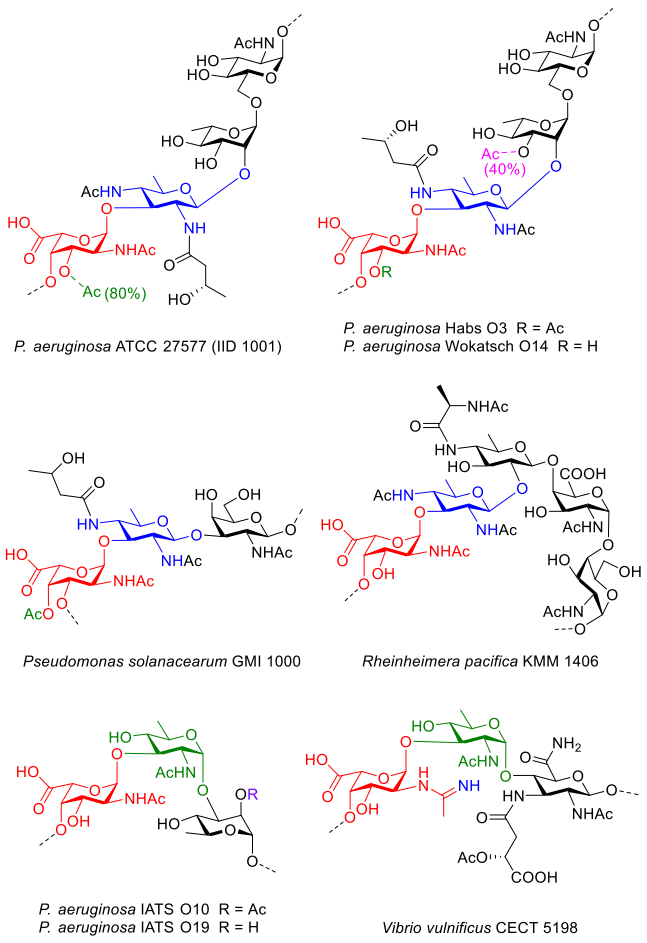

Figure 1. Structures of the LPS *O*-antigens of *P. aeruginosa* ATCC 27577, O10, and O19 as well as some other representative bacterial glycans containing the α-L-GalNAcA-(1 → 3)-β-D-Bac/α-D-QuiNAc disaccharides or similar motifs.

that of *Pseudomonas solanacearum* GMI 1000,[26] *Rheinheimera. pacifica* KMM 1406,[27] *Vibrio vulnificus* YJ016,[28] *Ralstonia pickettii*,[29] and *Alteromonas sp.* CMM 155.[30] The LPS *O*-antigens of *P. aeruginosa* IATS O10 and O19 (Figure 1), characterized by Knirel et al.,[9,31,32] are made up of the same repeating trisaccharide: 4-α-L-Gal*p*NAcA-(1 → 3)-α-D-Qui*p*NAc-(1 → 3)-α-L-Rha*p*-(1 →, whereas the O10 antigen contains an appendant acetyl group at the Rha 2-*O*-position. It is worth noting that structures similar to their nonreducing end disaccharide residue (labeled with red and green colors, Figure 1) are also present in the glycans derived from *V. vulnificus* CECT 5198 and *Pseudoalteromonas rubra* ATCC 29570.[33,34]

Herein, we reported the first total synthesis of oligosaccharides **1**−**4** (Figure 2) corresponding to the typical repeating units of the LPS *O*-antigens of *P. aeruginosa* ATCC 27577, O10, and O19, respectively. A linker with a free amino group was installed at the reducing end to enable further modification or conjugation with other molecules, making them very useful tools for biological and immunological studies of these *O*-antigens. Synthesis of **1**−**4** has involved several challenges. First, monosaccharides L-GalNAcA, D-Bac, and D-QuiNAc are rare amino sugars not commercially available. Second, the stereoselective construction of several 1,2-*cis*-glycosidic linkages in these structures is difficult, and related glycosylation reactions need to be prudently performed. Third, an array of functional groups, such as acetoxy, acetylamino, carboxyl, 3-

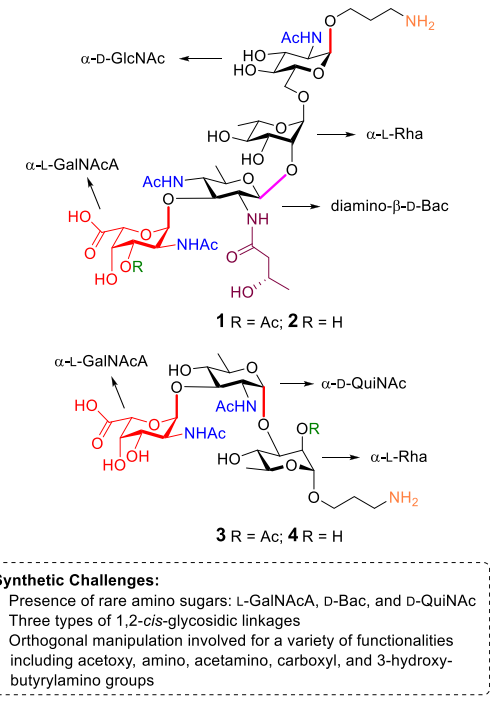

Figure 2. Structures of target oligosaccharides **1**−**4** and the involved synthetic challenges.

hydroxy-butyrylamino, and free amino groups, are present in the target compounds. Accordingly, a judicious choice of orthogonal protections and the reaction sequence is critical for the successful synthesis. To date, there has been no reported synthesis of these molecules or any oligosaccharides containing the α-linked L-GalNAcA unit. The synthetic methods established in the present work would be useful for the preparation of other similar oligosaccharides, such as those outlined in Figure 1 and their analogues.

RESULTS AND DISCUSSION

Retrosynthetic Analysis of Target Molecules 1−4

As outlined in Scheme 1, for compounds **1** and **2**, since uronic acids as glycosyl donors typically possess low reactivity and give poor stereoselectivity for glycosylations,[35] the L-GalN$_3$ would be employed as a precursor of the L-GalNAcA unit for glycosylation, which would be converted into the desired L-GalNAcA later. This postglycosylation oxidation strategy resulted in the intermediate **5** as the fully and appropriately protected form of **1** and **2**, and the orthogonal protection of **5** could enable late-stage acylation of the Bac 2-*N*-position after the glycosylation between trisaccharide **7** and L-GalN$_3$ donor **6**. In turn, **7** could be sequentially assembled from monosaccharides **8**−**10** and alcohol **11**. The nonparticipating azido groups at the C2-position of **6** and **10**, serving as the protected amino groups, could facilitate stereoselective 1,2-*cis*-glycosylations. Besides, the azido group at the C4-position of **8** could be utilized to differentiate its C2 amino group. The azido groups are relatively stable during the glycosylation and oxidation processes and could be readily converted into the desired acetylamino groups through convenient selective reduction and *N*-acetylation. The glycosylations using donors **8** and **9** were expected to be 1,2-*trans*-stereoselective due to the participation effects of the acyl groups protecting their 2-*N*/*O*-positions. The amino group in linker **11** was protected with

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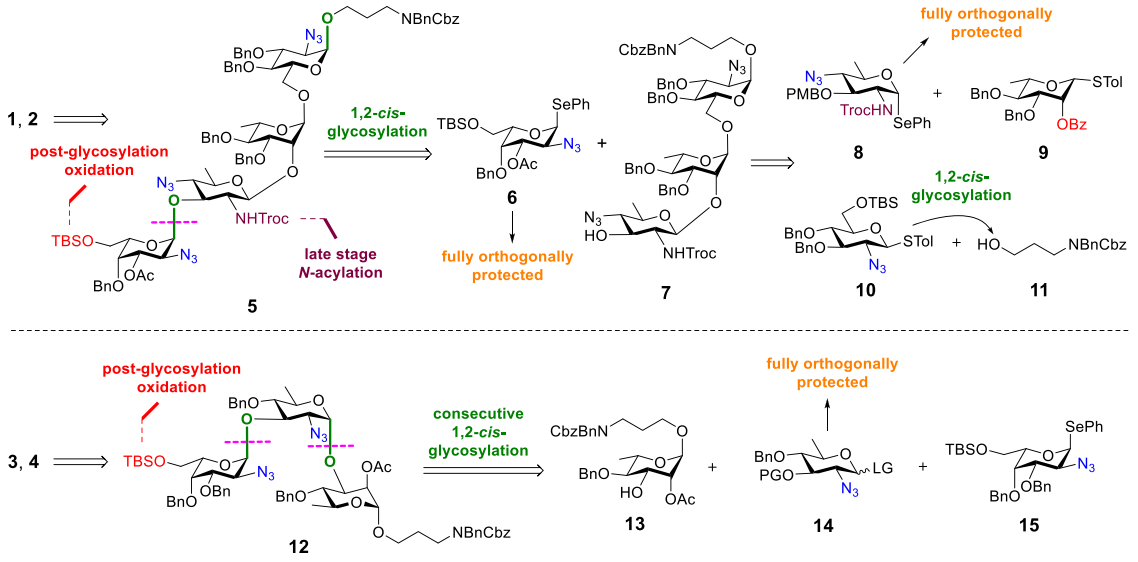
Scheme 1. Retrosynthetic Analysis of Target Molecules 1−4



the benzyl (Bn) and benzyloxycarbonyl (Cbz) groups to discriminate it from other amino groups. Likewise, synthetic targets **3** and **4** could be obtained from the properly protected **12** via a postglycosylation oxidation strategy and functional group interconversions. Moreover, it is worth noting that the monosaccharides in building blocks **6**, **8**, **14**, and **15** are rare amino sugars that need to be prepared in the lab.

Synthesis of Monosaccharide Building Blocks

Large-scale synthesis of the aforementioned properly functionalized monosaccharides as key building blocks was a prerequisite for the accomplishment of the target molecules. According to Scheme 2A, several 2-azido-derivatives **6**, **15**, **20**, and **21** of L-GalN_3 with different substituents at their 3,4-*O*-positions were prepared first and used as glycosyl donors to explore the best reaction conditions and to study the compatibility of α-selective glycosylation later on. After installation of the L-GalN_3 unit to oligosaccharides using these donors, their 6-*O*-TBS groups could be selectively removed to deliver a primary hydroxyl group, which could be readily oxidated to give the desired uronic acid. Deacetylation of **16**, which was obtained from D-galactose via a series of slightly modified literature procedures,[36−38] and regioselective silylation of the resultant C6-OH with *tert*-butyldimethylsilyl chloride (TBSCl) generated diol **17** in 82% overall yield. Stannylene acetal-directed benzylation or (2-naphthyl) methylation of **17** was regioselective for the 3-*O*-position to furnish **18** and **19**, respectively. Acetylation of the remaining C4-OH generated L-GalN_3 selenoglycoside **20** and **21** in high yields. Benzylation of the C4-OH in **19** was followed by replacement of 3-*O*-Nap with an acetyl group via oxidative cleavage of the Nap group by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and subsequent *O*-acetylation with acetic anhydride to afford compound **6** in 66% overall yield. Besides, benzylation of diol **17** led to the other L-GalN_3 donor **15** in 91% yield.

En route to the D-Bac selenoglycoside **8** (Scheme 2B), since we planned to use an azido group to mask the C4-NH_2 functionality, the C2-azido of the known D-FucN_3 derivative **22**[39] was reduced at early stage by using 1,3-propanedithiol, which was followed by protection of the resultant amino group with 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) to achieve the required orthogonality, leading to **23** in 79% yield for two steps. Thereafter, the axial C4-OH in **23** was converted into the corresponding triflate using triflic anhydride (Tf_2O) and pyridine, and subsequent substitution with an azide nucleophile from the equatorial face gave the fully orthogonally protected D-Bac donor **8**.

For the linker-equipped L-Rha derivative **13** (Scheme 2C), tin-mediated regioselective *p*-methoxybenzylation of the C3-OH in **24**[40] and acetylation of the C2-OH resulted in **25**. Next, glycosylation of **11** with **25** in the presence of *N*-iodosuccinimide (NIS) and triflic acid (TfOH) afforded **26** in a high yield of 89% and α-stereoselectivity ($J_{1H,1C}$ = 175 Hz). Thereafter, the cleavage of the PMB group with 10% TFA in CH_2Cl_2 smoothly generated **13**. Meanwhile, tin-mediated regioselective benzylation of **24** and benzoylation of the remaining C2-OH was straightforward to yield thioglycoside **9**.

Furthermore, different glycosyl donors **14a−d** of D-QuiN_3 with an ether or acyl group at 3-*O*-position were prepared and utilized to study the corresponding 1,2-*cis*-glycosylation later on (Scheme 2D). At first, diol **30** was readily obtained from the known **28**[41] through a series of transformations including protection of the C6-OH with *p*-toluenesulfonyl choride (TsCl), masking of C2,3-OHs with dimethoxypropane, benzylation of the left C4-OH, reduction with lithium tetrahydridoaluminate ($LiAlH_4$), and subsequent ketal deprotection with *p*-toluenesulfonic acid (TsOH). Thereafter, regioselective *p*-methoxybenzylation of the C3-OH using a tin-mediated method was followed by triflation and S_N2 substitution via a similar method used for **8** to furnish **14a**, a fully orthogonally protected thioglycoside donor of D-QuiN_3, in a 73% overall yield. Next, **14a** was converted to the trichloroacetimidate **14b** and Yu's donor **14c** via hydrolysis of the thioglycoside in the presence of *N*-bromosuccinimide (NBS) and then reaction of the resultant hemiacetal with trichloroacetonitrile or *N*-phenyl trifluoroacetimidoyl chloride, respectively, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Besides, thioglycoside **32** containing a remote participating levulinoyl (Lev) group at the 3-*O*-position was prepared from **14a** through the removal of the PMB ether with 10% TFA in CH_2Cl_2 and condensation of the resulting C3-OH with levulinic acid and 1-ethyl-3-(3-(dimethylamino)propyl)-

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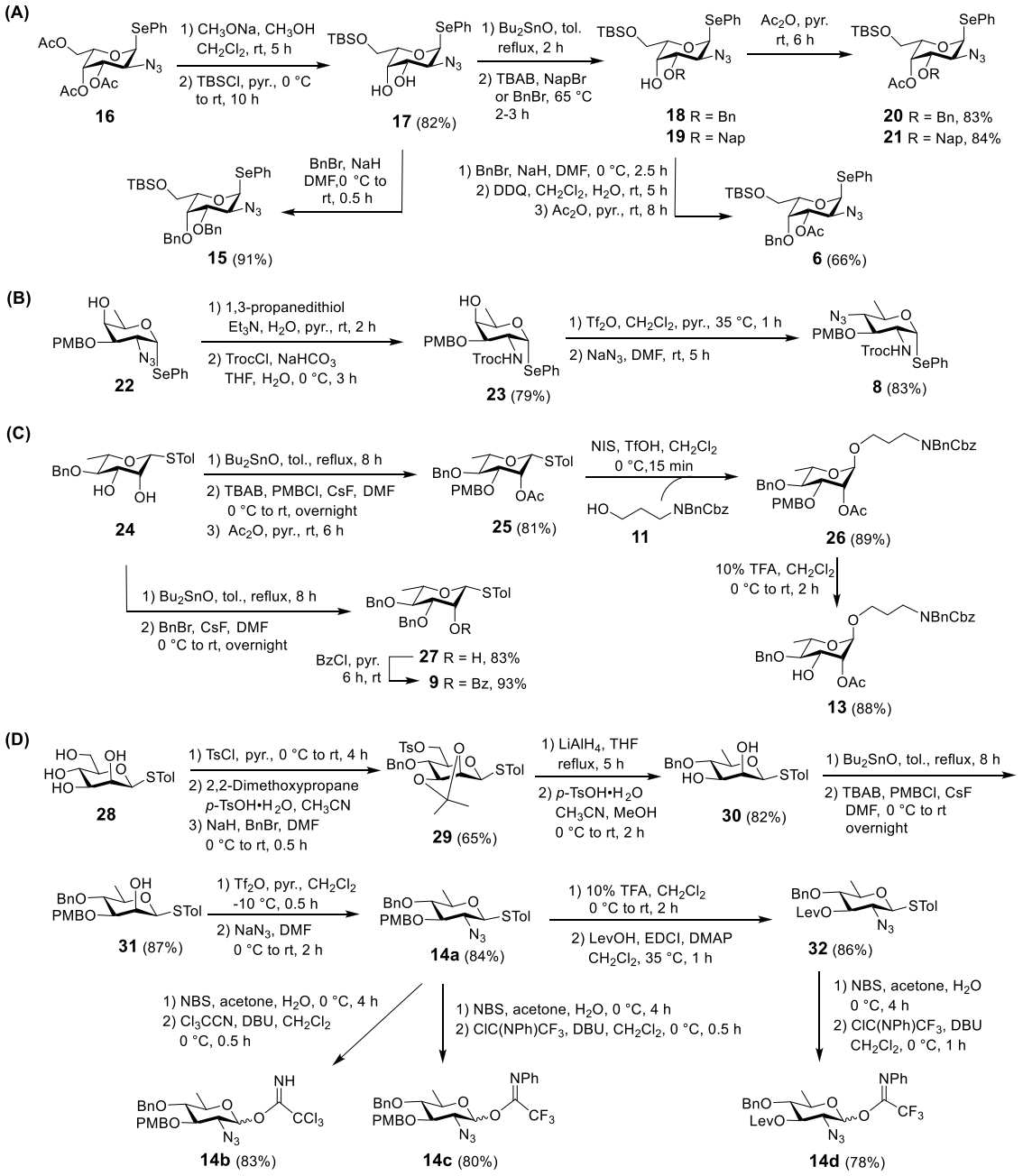
Scheme 2. Synthesis of Monosaccharide Derivatives of L-GalN (A), D-Bac (B), L-Rha (C), and D-QuiN (D)



carbodiimide (EDCI). Following the similar synthetic procedures used for **14c**, **32** was readily converted into another Yu's donor **14d**[42] in 78% yield for two steps.

Stereoselective Synthesis of Trisaccharide Acceptor 7

With all of the needed monosaccharide building blocks in hand, we then carried out the stereoselective synthesis of trisaccharide acceptor **7** (Scheme 3). The coupling reaction between the $GlcN_3$ donor **10** and *N*-Bn-*N*-Cbz-3-amino-propan-1-ol acceptor **11** was conducted in diethyl ether by using our previously developed α-glycosylation method[43−46] and, as expected, the α-linked compound **33** ($H\text{-}1^{GlcN}$, δ 4.78 ppm, s) was obtained in 72% yield together with small amount of the β-isomer (α/β = 8:1). The high α-selectivity of **33** was possibly attributed to the combined α-directing effects of ether solvent and the steric β-shielding property. After activation of donor **10** with *p*-toluenesulfenyl chloride (*p*-TolSCl) and silver triflate (AgOTf) promotion system, initially developed by Ye and Huang groups for oligosaccharide synthesis,[47−50] diethyl ether known as an α-directing solvent could act as a nucleophile to result in double S_N2 attacks at the anomeric carbon of the α-glycosyl triflate intermediate, leading to α-linked product preferentially.[51] Besides, the bulky 6-*O*-TBS group in **10** could exhibit the steric β-shielding property on the anomeric carbon to hinder the approach of acceptor from the β-face of the pyranose ring, also favoring the formation of α-glycoside.[43,45,52]

Thereafter, removal of the TBS group with $3HF{\cdot}Et_3N$ furnished the linker-equipped acceptor **34**, which was glycosylated with the L-Rha donor **9** under the promotion of NIS and trimethylsilyl trifluoromethanesulfonate (TMSOTf) to afford the disaccharide **35** in 78% overall yield and with α-

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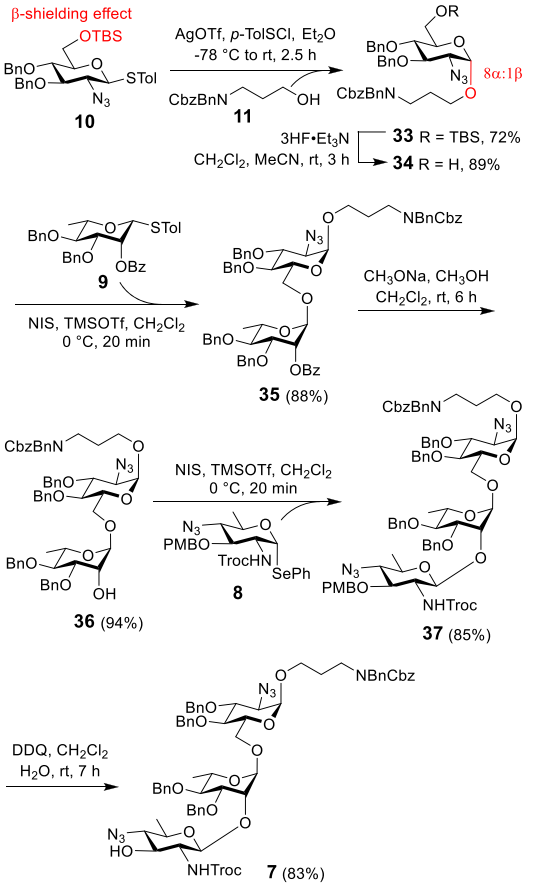
Scheme 3. Stereoselective Synthesis of Trisaccharide Acceptor 7



selectivity due to the neighboring participation effect of the donor 2-*O*-Bz group. Deprotection of the temporary Bz group under Zemplén conditions yielded **36**, and the exposed axial C2-OH was then coupled with the D-Bac selenoglycoside donor **8** also in the presence of NIS/TMSOTf, generating trisaccharide **37** in an 80% yield for two steps. Next, treatment of **37** with DDQ to remove the 3″-*O*-PMB delivered the trisaccharide acceptor **7**, ready for subsequent introduction of the L-GalN unit. Meanwhile, the β-configuration of the newly formed glycosidic bond of the D-Bac unit was more easily characterized by the ^{1}H NMR of **7** (Bac, H-1, δ 4.20 ppm, d, J = 8.4 Hz), and the 1,2-*trans*-selectivity should be attributed to the anchimeric assistance of the donor 2-*N*-Troc group.

Stereoselective α-Glycosidation of L-GalN$_3$

Next, we turned our attention to constructing the α-glycosyl linkage of L-GalN$_3$ with a high stereoselectivity. Previously, Yin[53] and van der Marel[37] group have prepared several L-GalN$_3$ donors and achieved the β-L-GalN$_3$ linkages in the context of monosaccharide and trisaccharide, respectively. However, to our knowledge, there has been no reported synthesis of oligosaccharides containing the α-L-GalNAcA residue, although it is widely present in bacterial glycans. To study the stereoselective α-glycosidation of L-GalN$_3$ in detail, we first designed and prepared compound **38**[54] (Table 1) as the model acceptor to study related glycosylations since the labeled skeleton of **38** (with blue color) could potentially mimic certain structural fragments of natural glycans in Figure 1. Besides, the L-GalN$_3$ selenoglycoside **15** was chosen as a model glycosyl donor because its C3,4-OHs were both protected with Bn groups so as to preclude the potential effect of remote group participation on resultant stereoselectivity.

The results are given in Table 1. The stereochemical outcomes of the condensation between **15** and **38** under the promotion of NIS/TfOH were revealed to be solvent- and, to some extent, temperature-dependent. The related disaccharide **40** was generated in 65% yield and low stereoselectivity (α:β = 3.5:1, Table 1, entry 1) in CH_2Cl_2 at 0 °C. Changing the solvent to a mixture of CH_2Cl_2 and Et_2O (1:1, v/v) gave a higher α-selectivity (α:β = 8:1, Table 1, entry 2). Using the same solvent system, the coupling reaction performed at −10 °C gave excellent α-selectivity and high yield (α:β > 15:1, 75%, Table 1, entry 3), and meanwhile, the same reaction at −40 °C also resulted in a high α-specificity (α:β = 11:1, Table 1, entry 4). Next, we tested the substrate scope of the α-glycosylation reactions. Under the optimized conditions, glycosylation of **38** with donor **20** containing an acetyl group at the axial 4-*O*-position afforded disaccharide **41**, as expected, with high yield and α-selectivity (α:β = 10:1, 81%, Table 1, entry 5). Moreover, the coupling reaction between **20** and a complex acceptor **39** containing a β-linked D-Bac unit (its preparation see the Supporting Information), representing a disaccharide fragment of *P. aeruginosa* ATCC 27577 *O*-antigen,[24] proceeded smoothly to afford the desired trisaccharide **42** in an 80% yield and α-only selectivity (Table 1, entry 6). Similarly, condensation between L-GalN$_3$ donor **21**, which contained a larger Nap group at the 3-*O*-position, and disaccharide acceptor **39** delivered the desired trisaccharide **43** again with good yield and exclusive α-specificity (Table 1, entry 7). Furthermore, we also tested the other L-GalN$_3$ donor **6** that contained a 3-*O*-Ac group. Delightfully, its coupling with mono- and disaccharides, **38** and **39**, gave excellent results including high yields and high (α:β = 12:1) to exclusive α-selectivity, furnishing the desired compounds **44** and **45**, respectively (Table 1, entries 8 and 9). The azido groups in products **40**−**45** can be facilely converted into the commonly existing acetamino groups via chemoselective azido reduction and *N*-acetylation, and the L-GalNAc unit can be converted into the L-GalNAcA via a postglycosylation oxidation strategy, as demonstrated in the synthesis of the target and highly functionalized tri- and tetrasaccharides described below.

It is worth noting that the solvent- and, to some degree, temperature-dependent manner of the above glycosylation method for α-L-GalN$_3$ was compatible with a variety of orthogonal protections, thus facilitating regioselective modification of resultant compounds, which would be useful for the synthesis of other similar oligosaccharides. Previously, several groups have achieved the α-D-GalN$_3$ linkage with excellent stereoselectivity by using different glycosylation methods, such as those based on ether solvent effect,[45,55] steric β-shielding effect of donor 4,6-*O*-di-*tert*-butylsilylene (DTBS) group,[56] as well as the combined influence of reagent modulation and remote participation effects of donor 3,4-*O*-acyl groups.[57] Ishida and co-workers[58] have also demonstrated that the high α-selective L-galactosylation could be achieved by using the DTBS-protected L-Gal donor. Besides, the α-L-FucN$_3$ linkage has been constructed with high stereoselectivity by utilizing several α-favoring factors including the combination of ether solvent and α-directing additive,[59] electron-donating protecting groups on the donor,[60] and the stereoelectronic property of donor 4-*O*-acyl functionality.[61] Probably, some of these stereodirecting effects could be utilized to develop a new

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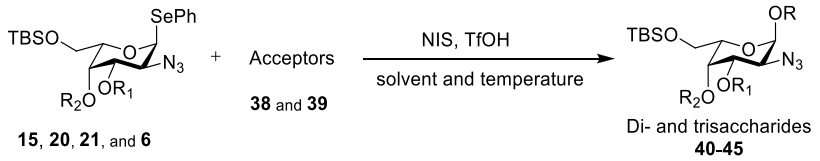
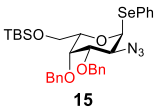
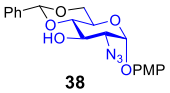
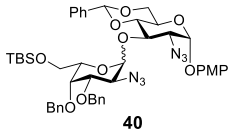
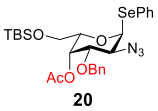
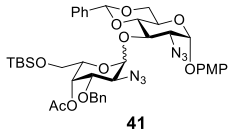
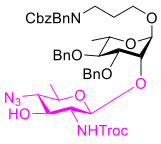
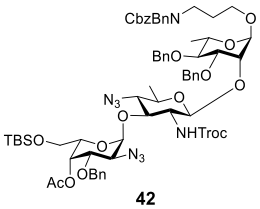
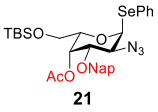
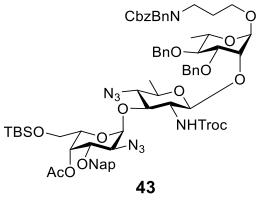
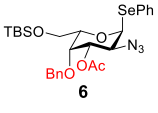
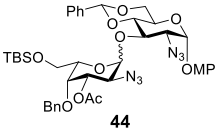
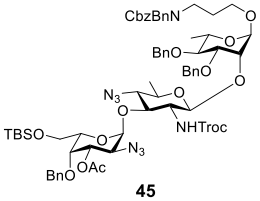
Table 1. Investigation of the Stereoselective α-Glycosidation of L-GalN$_3$



Entry	Donor	Acceptor	Product	Solvent and temperature	Yield[a] (α:β)[b]
1	 **15**	 **38**	 **40**	CH_2Cl_2, 0 °C	65% (3.5:1)
2	**15**	**38**	**40**	CH_2Cl_2:Et_2O (1:1), 0 °C	72% (8:1)
3	**15**	**38**	**40**	CH_2Cl_2:Et_2O (1:1), -10 °C	75% (> 15:1)
4	**15**	**38**	**40**	CH_2Cl_2:Et_2O (1:1), -40 °C	71% (11:1)
5	 **20**	**38**	 **41**	CH_2Cl_2:Et_2O (1:1), -10 °C	81% (10:1)
6	**20**	 **39**	 **42**	CH_2Cl_2:Et_2O (1:1), -10 °C	80% (α-only)
7	 **21**	**39**	 **43**	CH_2Cl_2:Et_2O (1:1), -10 °C	81% (α-only)
8	 **6**	**38**	 **44**	CH_2Cl_2:Et_2O (1:1), -10 °C	82% (12:1)
9	**6**	**39**	 **45**	CH_2Cl_2:Et_2O (1:1), -10 °C	83% (α-only)

[a]Isolated yield. [b]^{1}H NMR analysis of the reaction mixture.

synthetic methodology for constructing the α-L-GalN$_3$ linkage in the future.

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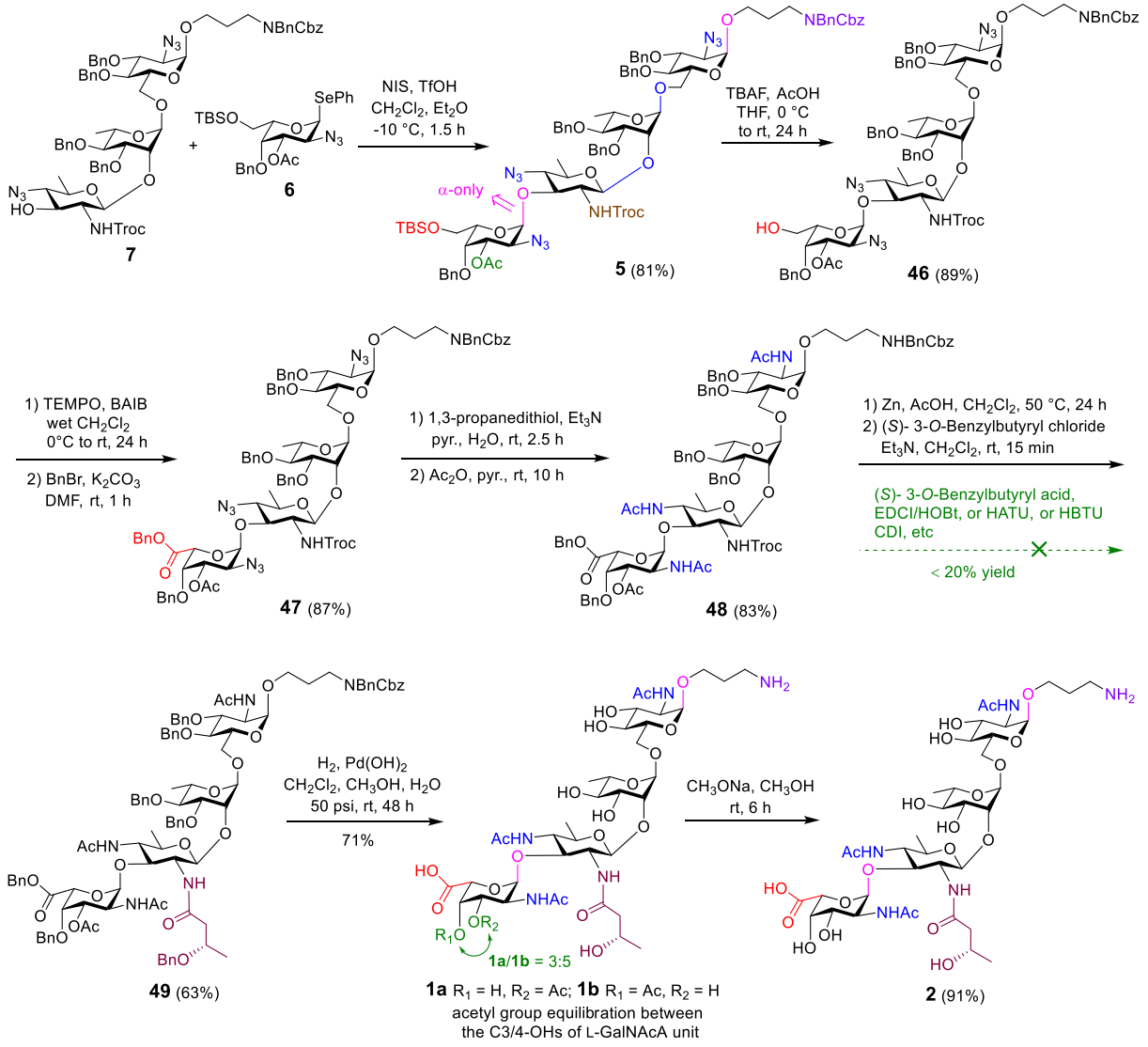
Scheme 4. Synthesis of the *P. aeruginosa* ATCC 27577 Tetrasaccharide Haptens 1a,b and 2



Synthesis of the *P. aeruginosa* ATCC 27577 Tetrasaccharide Haptens 1a,b and 2

After the 1,2-*cis*-glycosylation method of L-GalN$_3$ was established, we then applied it to continue the synthesis of **1** and **2** (Scheme 4). Under the optimized conditions, glycosylation of **7** with **6** proceeded smoothly to generate tetrasaccharide **5** in an 81% yield and α-only selectivity, while the newly created α-glycosyl linkage was clearly verified by the small anomeric coupling constants ($J_{H1''',2'''}$ = 3.0 Hz) in its ^{1}H NMR spectrum. The appropriately designed **5** contained a series of orthogonal protections masking the sites of desired functionalities, and their handling sequence needs to be carefully tackled. After a detailed evaluation of the properties of these protecting groups and the expected resultant functionalities as well as the corresponding reaction conditions, we decided to first carry out the oxidation reaction to get the desired uronic acid motif.

Accordingly, the 6‴-*O*-TBS in **5** was removed by using tetrabutylammonium fluoride (TBAF) and HOAc to liberate the nonreducing end primary hydroxyl group. Oxidation preparation of uronic acid in the context of complex oligosaccharides was typically difficult and sometimes led to target compounds with low yields.[62,63] Recently, we have developed a modified 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO)/(diacetoxyiodo)benzene (BAIB) oxidation-protocol, based on which complex *V. vulnificus* oligosaccharides containing the α-D-GalNA unit have been achieved.[55] To our delight, this TEMPO/BAIB protocol was also efficient for the preparation of the α-L-GalNA contained molecule, and the benzyl uronate **47** was obtained in a high 87% overall yield after in situ benzylation of the carboxylic acid intermediate. Next, three azido groups in **47** were simultaneously converted into the acetylamino groups via two steps including reduction of the azido groups with 1,3-propanedithiol and acetylation of the resultant amino groups with acetic anhydride, leading to compound **48** in 83% yield. However, subsequent acylation of the Bac 2-*N*-position turns out to be problematic. Although cleavage of the 2″-*N*-Troc of **48** with Zn/HOAc was carried out smoothly, the followed *N*-amidation reaction using (*S*)-3-*O*-benzylbutyryl acid did not give the desired **49** in acceptable yield. In the presence of different condensation reagents, such as EDCI/HOBt, HATU, HBTU, CDI, etc., all the reactions were not efficient, and in the best case, compound **49** was generated with <20% low yields. Since some carboxylic acids

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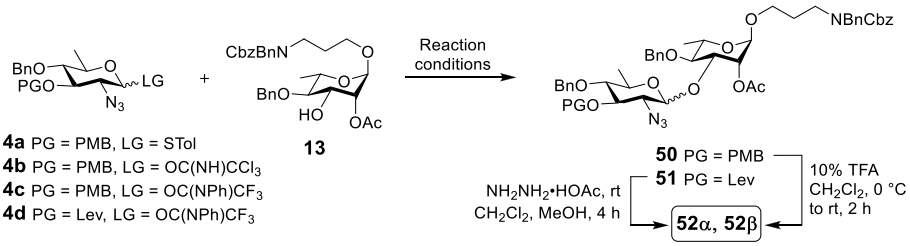
Table 2. Investigation of the Stereoselective α-Glycosidation of D-QuiN$_3$



entry	donor	solvent	activation methods	yield[a] (α:β)[b]
1	14a	CH_2Cl_2:Et_2O (1:1)	NIS, TfOH, 0 °C	87% (1:1)
2	14a	CH_2Cl_2:Et_2O (1:1)	NIS, TfOH, −20 °C	85% (1.2:1)
3	14b	CH_2Cl_2:Et_2O (1:3)	TMSOTf, −40 °C	81% (1.3:1)
4	14b	CH_2Cl_2:Et_2O (1:3)	TMSOTf, thiophene, −40 °C	80% (1.3:1)
5	14b	CH_2Cl_2:Et_2O (1:3)	TMSOTf, thiophene, 0 °C	83% (1.8:1)
6	14c	CH_2Cl_2	TfOH, DMF, −78 to 0 °C	82% (5:1)
7[42]	14d	CH_2Cl_2	TfOH, DMF, −78 to 0 °C	72% (>15:1)

[a]Isolated yield. [b]^{1}H NMR analysis of the reaction mixture.

have been successfully employed to acylate the amine group on different sugar substrates,[64,65] we supposed that the above failure for **49** was possibly attributed to the complex structure of a tetrasaccharide substrate and the ensued steric hindrance for the Bac C2-NH_2. To overcome this issue, the alternative and more reactive (*S*)-3-*O*-benzylbutyryl chloride[59] was utilized to perform the amidation reaction and to our delight, the coupling reaction carried out smoothly to afford **49** in a high 63% yield for two steps after flash silica gel chromatography.

Thereafter, **49** was subjected to $Pd(OH)_2$-catalyzed hydrogenation in a mixed solvent to remove all of the Bn and Cbz groups. HR MS analysis of the reaction mixture indicated that target tetrasaccharide **1** (m/z 956.4185 for $[M + H]^+$) was generated after 48 h. However, detailed analysis of its 1D and 2D NMR spectra revealed the partial migration of the acetyl group to the adjacent 4-*O*-position of the L-GalNAcA unit, leading to a 4‴-*O*-acetylated **1b** (**1a**/**1b** = 3:5). All attempts to separate the regioisomers including the high-performance liquid chromatography (HPLC)-separation on a C18 column were failed. Besides, for the newly generated crude **1** that was facilely prepared by filtration of the catalyst and concentration *in vacuo*, its ^{1}H NMR spectrum immediately recorded after dissolving in D_2O also revealed the same equilibrium between **1a** and **1b**. This finding indicated that the isomerization should occur during the hydrogenation process. Performing the hydrogenation in an acidic environment[66] (adding HCl or HOAc) did not suppress the acetyl group migration effectively. Consequently, we proposed that the acetyl migration was probably attributed to their inherent structures. It is worth noting that the *O*-acetyl group migration between neighboring hydroxyl groups and even across the glycosidic bond to different saccharide units was a common phenomenon in oligo- and polysaccharides.[67−70] Since 3-*O*-acetylation of the L-GalNAcA unit is nonstoichiometric in the natural LPS *O*-polysaccharides of *P. aeruginosa* ATCC 27577, we also obtained the deacetylated antigen **2** by treatment of **1a**,**b** with CH_3ONa. After a G15 column purification and lyophilization, **2** was generated in 91% yield, and its structure was extensively verified by the 1D and 2D NMR and HR MS data.

Synthesis of the *P. aeruginosa* O10 and O19 Trisaccharide Haptens 3 and 4

Based on the above accomplishment, we next turned our attention to the synthesis of *P. aeruginosa* O10 hapten **3** and O19 hapten **4**. Initially, stereoselective α-glycosidation of the D-QuiN$_3$ using different donors was extensively studied since the α-D-QuiN residue also widely existed in natural glycans, and the results are listed in Table 2. Glycosylation of thioglycoside donor **14a** with linker-equipped Rha acceptor **13** under the promotion of NIS/TfOH in a mixed solvent (CH_2Cl_2:Et_2O, v/v, 1:1) at 0 °C gave disaccharide **50** in 87% yield but with no stereoselectivity (α:β = 1:1, Table 2, entry 1). Lowering the temperature to −20 °C gave almost no improvement (α:β = 1.2:1, Table 2, entry 2). In addition, the coupling reactions using imidate donor **14b** in a mixed solvent (CH_2Cl_2:Et_2O, v/v, 1:3) under the activation of TMSOTf at different temperatures or even in the presence of thiophene, an α-directing additive,[71] did not afford an acceptable α-selectivity for **50**, albeit with good yields (Table 2, entries 3−5). Mong[72] and Codée groups[73] have revealed that the additive dimethylformamide (DMF) could direct glycosylation to result in a product with more α-specificity owing to its coordination with the anomeric oxocarbenium to form the more reactive β-glycosyl imidate intermediate. Thus, we conducted the coupling reaction using donor **14c** in CH_2Cl_2 under the promotion of TfOH with DMF, and **50** was generated in 82% yield and moderate stereoselectivity (α:β = 5:1, Table 2, entry 6). While we carried out these studies, the Yin group[42] reported an excellent 1,2-*cis* glycosylation method for stereoselective construction of the α-glycosyl linkage of D-QuiN$_3$ by using the combined α-directing effects of DMF and the remote participating assistance of donor 3-*O*-Lev group. By means of this method, they furnished the synthesis of a disaccharide analogue of **51**, just with a longer anomeric linker. Using the same reaction conditions and donor **14d**, we obtained disaccharide **51** with almost exclusive α-specificity (α:β > 15:1, Table 2, entry 7). The newly formed glycosyl linkages in **50** and **51** were more easily identified by the ^{1}H NMR spectra of the 3-*O*-deprotected products **52*α*** (QuiN$_3$, H-1, δ 5.02 ppm, d, J = 3.6 Hz) and **52*β*** (QuiN$_3$, H-1, δ 4.55 ppm, d, J = 7.2 Hz).

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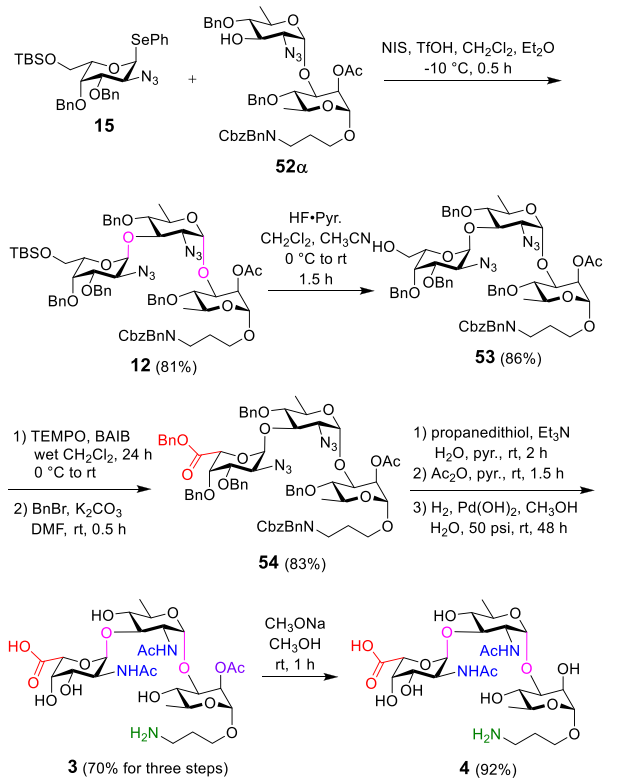
After getting the disaccharide acceptor **52*α***, we next performed its coupling with the L-GalN_3 donor **15** under the optimized glycosylation conditions described above (see Table 1), as shown in Scheme 5. Expectedly, the desired trisaccharide

Scheme 5. Synthesis of the *P. aeruginosa* O10 and O19 Trisaccharide Haptens 3 and 4



12 was generated in a high 81% yield and *α*-only selectivity (L-GalN_3, H-1, *δ* 5.62 ppm, d, *J* = 3.6 Hz). Cleavage of the 6″-*O*-TBS group with HF·Pyr was followed by oxidation of the exposed primary hydroxyl group also by means of our modified TEMPO/BAIB oxidation protocol[55] to produce compound **54** in 71% overall yield after benzylation of the carboxylic acid intermediate. Thereafter, chemoselective reduction of two azido groups in **54**, simultaneous *N*-acylation, and the succeeding $Pd(OH)_2$-catalyzed hydrogenation to deprotect all of the Bn and Cbz groups were carried out smoothly, delivering the target *P. aeruginosa* O10 hapten **3** in 70% overall yield for three steps. At last, the removal of the 2-*O*-Ac group in **3** with CH_3ONa afforded the *P. aeruginosa* O19 hapten **4**. The structures of **3** and **4** were verified extensively by the 1D and 2D NMR and HR MS data.

CONCLUSIONS

In summary, we achieved the first total synthesis of the repeating tetra- and trisaccharide units of the LPS *O*-antigens of *P. aeruginosa* ATCC 27577, O10, and O19. This work also represents the first synthesis of an oligosaccharide containing an *α*-L-Gal*p*NAcA residue, which is widely expressed in a variety of bacterial glycans. The target molecules were synthetically challenging due to the presence of several rare amino sugars, difficult 1,2-*cis*-glycosidic linkages, and an array of functionalities. Through careful design and implementation of orthogonal protection and deprotection tactics and detailed evaluation of various glycosylation and functionalization strategies, we overcame a series of challenges and obtained target antigens **1a**,**b** and **2**−**4** via a linear glycosylation strategy. All glycosyl linkages, including the difficult 1,2-*cis*-glycosidic bonds of amino sugars, were constructed in high yields and stereoselectivity. The synthesis is also highlighted by a postglycosylation oxidation strategy to convert neutral sugars into uronic acids, as well as late-stage *N*-acylation. Furthermore, efficient synthetic methods were developed for several rare amino sugars in target molecules. We have also discovered an acetyl group migration phenomenon during the synthesis of the *O*-acylated repeating unit of *P. aeruginosa* ATCC 27577 antigen. Overall, the synthetic strategy established here would be useful for the preparation of other similar oligosaccharides, especially those containing the *α*-L-GalNAcA-(1 → 4)-D-Bac/QuiNAc motif or its derivatives. Additionally, all the synthetic haptens contain a linker with a free amino group at the glycan-reducing end, which could facilitate further regioselective modifications and conjugation with other molecules for various biological studies and applications.

METHODS

General Procedure for Glycosylation of Donor 6/15/20/21 with Acceptor 38/39 using NIS/TfOH as a Promoter

The reaction solution of donor **6**/**15**/**20**/**21** (150 *μ*mol, 3.0 equiv), acceptor **38**/**39** (50.0 *μ*mol, 1.0 equiv), and freshly activated MS 4 Å in anhydrous solvent (2.0 mL) was stirred at room temperature for 1 h and then cooled to the specific temperature. NIS (43.9 mg, 0.195 mmol, 3.9 equiv) and TfOH (1.3 *μ*L, 15.0 *μ*mol, 0.3 equiv) were added under a N_2 atmosphere. The reaction mixture was stirred until TLC analysis indicated complete conversion of the starting material, then neutralized with Et_3N, diluted with CH_2Cl_2 (20 mL), and filtered. The filtrate was washed with saturated $Na_2S_2O_3$ solution and brine, dried over Na_2SO_4, and concentrated under a vacuum. A small portion of the residue was dissolved in $CDCl_3$ and analyzed by ^1H NMR to give the *α*:*β* anomeric ratio. The remaining residue was purified by silica gel column chromatography in an isolated yield.

General Procedures of the Modified TEMPO/BAIB Oxidation and Benzylation To Synthesize Compounds 47 and 54

To a stirred solution of each oligosaccharide **46** or **53** (1.0 equiv) in anhydrous CH_2Cl_2 (0.05 M) were added TEMPO (0.2 equiv) and BAIB (1.5 equiv) at 0 °C. The reaction mixture was warmed up to room temperature and afforded the aldehyde intermediate almost quantitatively in 4 h, at which time TLC analysis indicated complete conversion of the starting material. Subsequently, water (1% in CH_2Cl_2) and BIAB (3.0 equiv) were added at 0 °C, and the mixture was stirred vigorously at room temperature for about 20 h. TLC analysis (CH_2Cl_2/MeOH, 20:1 v/v) indicated complete conversion of the aldehyde intermediate. The reaction mixture was quenched with dropwise addition of a saturated $Na_2S_2O_3$ solution and diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated under vacuum to give a residue.

The crude residue was dissolved in DMF and mixed with BnBr (1.5 equiv) and K_2CO_3 (2.0 equiv) at room temperature. The reaction mixture was stirred under N_2 protection for 30 min and extracted with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by silica gel column chromatography with EtOAc and toluene as the eluent to give the desired uronate **47** or **54**.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacsau.4c00321.

Experimental procedures, analytical data, and NMR and MS spectra of all synthetic compounds (PDF)

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#X.Y., H.Z., and Q.Z. contributed equally. CRediT: **Xiaoyu Yang** investigation, methodology; **Han Zhang** funding acquisition, investigation, writing-original draft; **Qingpeng Zhao** investigation; **Qingjiang Li** visualization; **Tiehai Li** formal analysis, visualization; **Jian Gao** funding acquisition, project administration, writing-review & editing.

Notes

The authors declare no competing financial interest.

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