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Total Synthesis of *Pseudomonas aeruginosa* 1244 Pilin Glycan via *de novo* Synthesis of Pseudaminic Acid.

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ABSTRACT: Pseudaminic acid (Pse) is a nonulosonic acid unique to bacterial species, found as a component of important cell surface glycans and glycoproteins in various pathogenic species, such as the critical hospital threat *Pseudomonas aeruginosa*. Herein we present the development of a facile and scalable *de novo* synthesis of Pse and its functionalized derivatives from easily available Cbz-L-*allo*-threonine methyl ester (16 steps in 11% yield). The key reactions in our *de novo* synthesis involve the diastereoselective glycine thioester isonitrile-based aldol-type reaction to create the 1,3-*anti*-diamino skeleton, followed by the Fukuyama reduction and the indium-mediated Barbier-typed allylation. Moreover, we have studied the glycosylation of the Pse glycosyl donors and identified the structural determinants for its glycosylation diastereoselectivity, which enabled us to complete the total synthesis of *P. aeruginosa* 1244 pilin trisaccharide α-5NβOHC$_4$7NFmPse-(2→4)-β-Xyl-(1→3)-FucNAc.

1. INTRODUCTION

Protein glycosylation is a common and essential posttranslational modification in eukaryotes that significantly enriches the functional and structural diversity of proteins. Recently, bacterial protein glycosylation has emerged as a new entry to study bacterial pathogenesis and develop novel therapeutic intervention.[1-4] In particular, bacterial proteins glycosylated with pseudaminic acid (Pse) **1** are intriguing (Figure 1a). Pse was first discovered by Knirel et al. from the O-antigen of the LPS of *Pseudomonas aeruginosa* O7/O9 and *Shigella boydii* type 7 in 1984.[5] Different from eukaryotic sialic acid **2** (Figure 1b), Pse contains one more amido group (on *C*7) and one less hydroxyl group (on *C*9), together with the opposite chirality of *C*5, *C*7, and *C*8. The diverse substitutions on the two amino groups, varying from acetyl to 3-hydroxybutyryl and formyl groups, further increase the structural complexity. Pse and its naturally occurring derivatives are unique to bacterial species, and they have been found as components of important cell surface glycans (e.g., LPS and capsular polysaccharide) and glycoproteins (e.g., pilin and flagellin) in various pathogenic species.[6] In glycoproteins, Pse is usually linked to the peptide backbone via Ser/Thr (e.g., in *H. pylori* and *C. jejuni*), or via another glycan(s) (e.g., in *P. aeruginosa*). Considering the importance of sialic acid in eukaryotes, Pse is expected to play critical roles in bacteria.[7] However, the exact biological function of Pse-containing glycans in bacterial glycoproteins remains elusive, which is partially due to the lack of highly homogeneous Pse-containing samples. Thus, the development of an efficient synthesis of Pse and its derivatives will be of great value. The synthetic approach could provide important chemical biology tools to investigate proteins or enzymes that utilize this glycan, and to perform systematic studies to clarify the function of bacterial protein glycosylation.

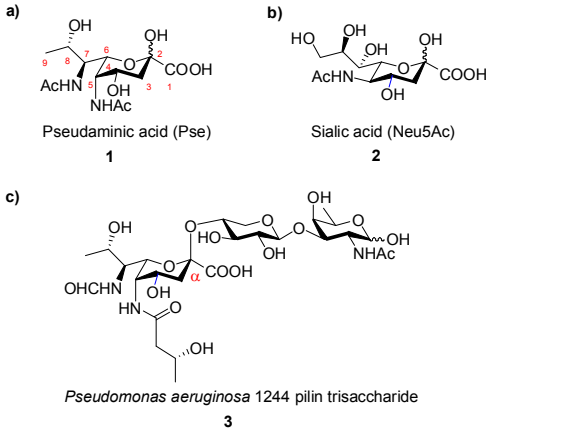


FIGURE 1. Structures of pseudaminic acid, sialic acid, and *P. aeruginosa* 1244 pilin trisaccharide.

Among the Pse-containing glycans and glycoconjugates, the *P. aeruginosa* 1244 pilin glycan trisaccharide **3** (Figure 1c) attracted our attention. *Pseudomonas aeruginosa*, the key member of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobater* species), is a Gram negative pathogen notorious for its antibiotic resistance and biofilm formation.[8] Opportunistic infection of immunocompromised patients (e.g. cystic fibrosis) caused by *P. aeruginosa* is life threatening and becomes heavy burden for public health. *P. aeruginosa* 1244 strain, which is featured by the glycosylated pilin, is a clinical isolate that has been used in the pilus mediated adhesion studies.[9] The structure of the pilin glycan **3**, abbreviated as α-5NβOHC$_4$7NFmPse-(2→4)-β-Xyl-(1→3)-FucNAc, was elucidated by Castric et al. through NMR and ESI-MS analysis in 2001.[10] (In several review papers,[11–13] the structure was somehow cited incorrectly in the configuration of the Pse-Xyl glycosidic linkage, which

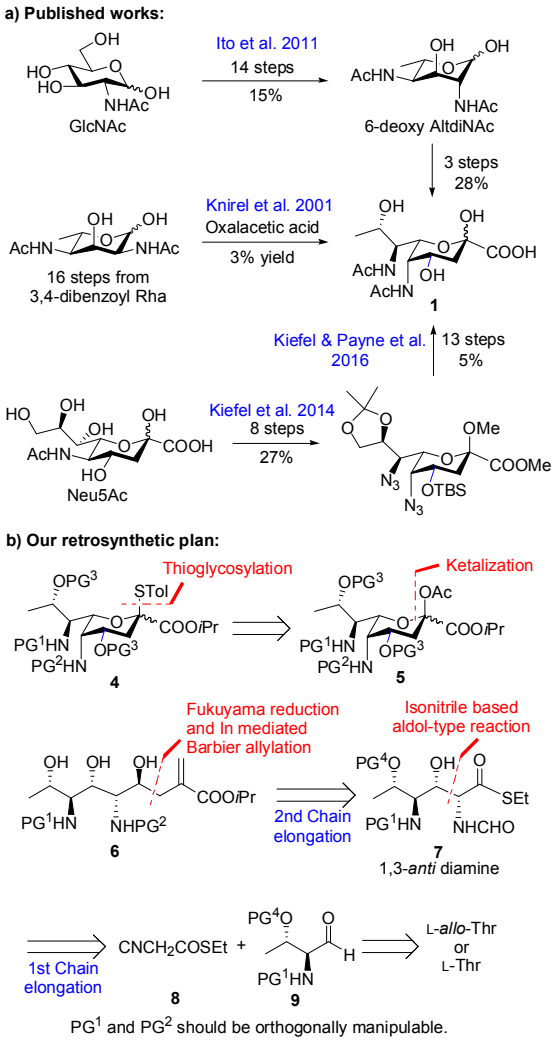
should be axial (α) not the equatorial (β)). The glycan **3** shares the same structure with the O-antigen repeating unit of the O7 serotype LPS from 1244 strain,[14] which suggests that the glycan originates from the same metabolic pathway as the O-antigen biosynthesis. The pilin in *P. aeruginosa* 1244 is glycosylated with trisaccharide **3** at the C-terminal Ser148 residue through β glycosidic linkage to the D-fucosamine.[15] This post-translational modification is mediated by the *pilO* gene product, which works as the oligosaccharyltransferase to mediate the transfer of the trisaccharide from the carrier lipid to pilin.[16] The pilin assembles in a highly organized manner to form pili, which are important virulence factors in the bacterial pathogenesis.[17] The pili are involved in the adhesion of the bacteria to the host tissue, and their extension and retraction is responsible for twitching motility of the bacteria.[18] As reported by Castric et al., the glycans were located evenly on the surface of the pili and significantly affected their hydrophilicity, while glycosylation had no influence on the piliation level, phage attachment, and twitching motility. Glycosylated *P. aeruginosa* 1244 behaved to colonize the lung tissue 3 times more efficiently than the non-glycosylated *P. aeruginosa* 1244.[19] In addition, the *P. aeruginosa* 1244 pilin glycan has been shown to play an important role in the immunogenicity. A pilin glycopeptide isolated from *P. aeruginosa* 1244 protected the immunized mice from challenge with *P. aeruginosa*, which illustrated the potential use of a trisaccharide **3** glycoconjugate in vaccine development.[20] For further biological and medicinal investigation, an efficient synthetic route towards **3** to provide adequate amount of sample with structural homogeneity is highly desired.

Herein, we report a feasible *de novo* synthesis of Pse **1** and related derivatives, and the first total synthesis of *P. aeruginosa* 1244 pilin trisaccharide **3** through α-selective glycosylation of the Pse glycosyl donors.

2. RESULTS AND DISCUSSION

2.1 Synthetic plan. From the structural complexity of trisaccharide **3**, the foreseeable challenges in the synthesis adventure include: (a) accessibility of the densely functionalized Pse moiety, for which the current syntheses were limited to prepare only the *N*-acetylated form; (b) the unknown reactivity of the Pse in the stereocontrolled glycosylation; (c) three amino groups on the trisaccharide carrying different functionalities (*N*5 and *N*7 on Pse and *N*2 on FucNAc) which would require orthogonal protections and careful manipulation. In particular, *cis* orientation of the hydroxyl group on *C*4 and the amino group on *C*5 could potentially cause undesired acyl group transfer or formation of cyclic carbamates.

SCHEME 1. Three published synthetic routes toward pseudaminic acid and the retrosynthetic plan of our *de novo* synthesis.



As the first step of our synthetic plan, the Pse derived glycosyl donor **4** with appropriate leaving groups and tunable protecting group patterns had to be synthesized in an efficient and scalable manner. Till now, few synthetic routes for **1** have been reported (Scheme 1a): Knirel's synthesis based on the biomimetic decarboxylative aldol reaction of oxalactic acid with 2,4-diacetamido-2,4,6-trideoxy-L-allopyranose (overall 3% yield);[21] Ito's synthesis involved the Barbier reaction of 6-deoxy-AltdiNAc (overall 4% yield);[22] Payne and Kiefel's synthesis relied on the Zbiral deamination of Neu5Ac (overall 1.4%).[23] More recently, the synthesis of nonulosonic acid species structurally related to Pse was reported by Crich.[24] All the above-mentioned approaches made Pse moieties chemically accessible and provided solid evidence for its structure. However, the acetyl groups on *N*5 and *N*7 in these products not only will impede the optimization of the glycosylation reaction via tuning of protecting groups on *N*5 (as investigated in the sialic acid chemistry), but also conflict with the synthesis of Pse-containing glycan with different *N*-functionalities, such as glycan **3** with *N*7-formyl and *N*5-3-hydroxybutyryl groups.

Confronted with these challenges, we conceived of the idea of a *de novo* synthesis of Pse derivatives carrying orthogonal functionalities on *N*5 and *N*7 for flexible manipulations from simple starting materials (Scheme 1b). Based on the chirality of *C*7 and *C*8, L-*allo*-threonine was chosen as the starting material. In the recent total synthesis of Legionaminic acid reported by Seeberger et al., D-threonine served successfully as

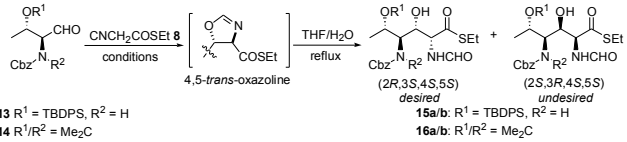
the source of chirality in the *de novo* synthetic approach.[25] The key strategy of our synthetic plan is the coupling reaction between the glycine thioester isonitrile **8**[26] and the aldehyde **9**, via *syn* aldol-type reaction to create a 1,3-diamino skeleton **7**, in which two new chiral centers would be simultaneously built. The absolute configurations of the generated chiral centers need to be determined and controlled. We anticipated that the protecting group of the aldehyde would affect the diastereoselectivity. The thioester introduced here could be then converted via the Fukuyama reduction into the aldehyde functionality for the subsequent indium-mediated Barbier-type allylation to give compound **6**.[27] Next, subsequent ozonolysis, ketalization of the ketoester, acetylation, thioglycosylation, and protecting group manipulation would produce the thioglycoside **4**.

2.2 *De novo* synthesis of Pse glycosyl donors. Our synthesis of Pse started from L-threonine (Scheme 2) whose *C*3 *R*-configuration was inverted to *S* to form L-*allo*-threonine (also commercially available). After installation of Cbz group and methyl ester, the intermediate **10** was obtained in > 50 g scale.

In our original attempt, the 3-hydroxyl group of **10** was protected by TBDPS group to give **11**, which was transformed to the corresponding aldehyde **13** via the DIBAL-H reduction. In the following aldol-type addition of isonitrile **8** to **13**, the oxazoline intermediate was formed smoothly under catalysis of Cu_2O,[28] followed by one-pot hydrolysis to give diastereomers **15a**/**15b** (1 : 10) in 72% yield. However, the configurations of the two newly formed chiral centers of the major product **15b** were determined to be undesired through the derivatization of the thioester **15b** to L-bacillosamine **31** (Scheme 3).

To reverse the diastereoselectivity of this coupling reaction, as listed in Table 1, different catalysts (weak bases and combinations of bases and Lewis acids) and solvents were screened, but without success (entries 1–13). Based on the analysis of the stereoselectivity model, the undesired diastereoselectivity was likely attributed to the bulky TBDPS group. Thus, the acetonide-protected ester **12** was next prepared and derived to the corresponding Garner-type aldehyde **14**. To our delight, in the subsequent aldol-type addition of **8**, 5.0 : 1 ratio favoring the desired diastereomer **16a** was obtained using $LiBF_4$/*i*Pr_2NEt combination in 1,2-dichloroethane (DCE)-DMF mixture (entry 20), while the undesired diastereomer **16b** was still favored under Cu_2O catatysis (entries 14–17). The configurations of the two newly formed chiral centers of the adducts **16a** and **16b** were determined via derivatization into L-2,4-diacetamido-2,4,6-trideoxyaltrose **35** and L-bacillosamine **31**, respectively (Scheme 3). It is worthy to note that addition of DMF was critical for solubilizing $LiBF_4$, while only 2.0 : 1 ratio was obtained in the suspension of $LiBF_4$ in DCE (entry 19). The obtained diastereoselectivity under such condition can be rationalized by the lithium cation chelation stabilized Felkin-Anh model.

TABLE 1. Optimization of the diastereoselective addition of isonitrile 8 to aldehdyes.

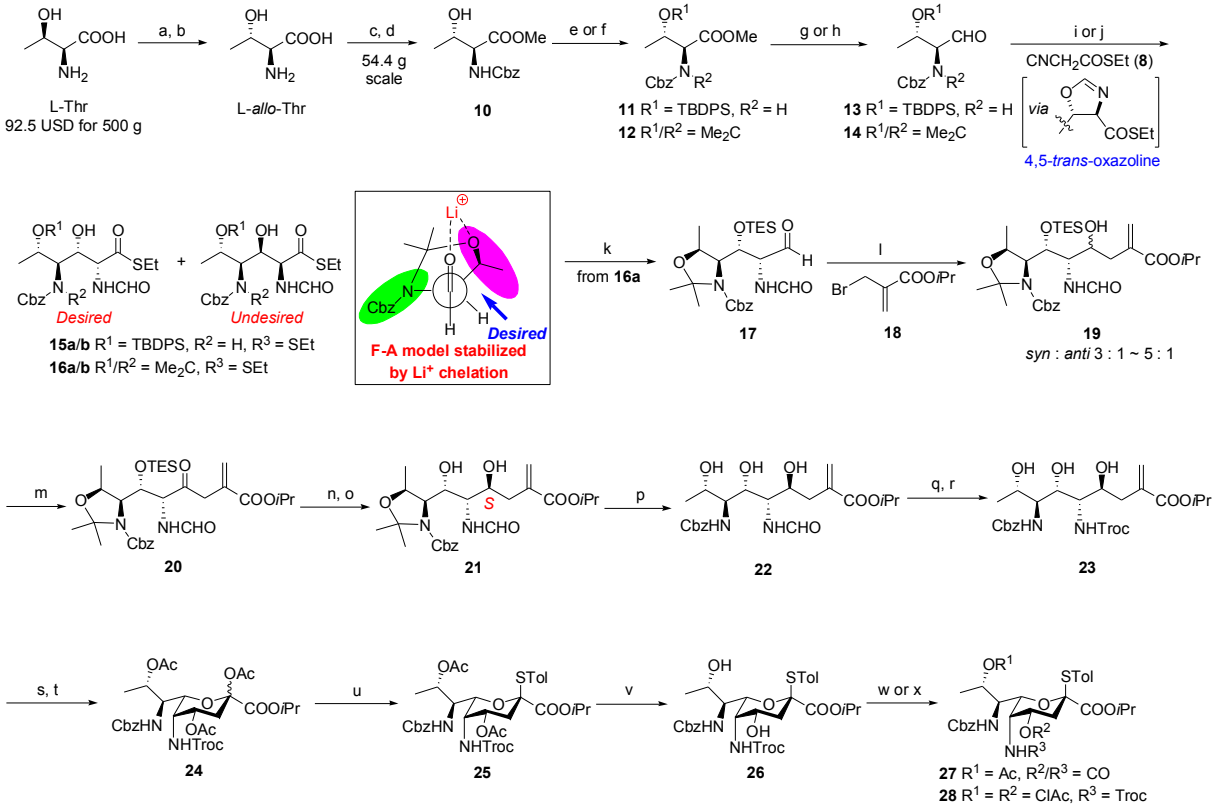


13 R^1 = TBDPS, R^2 = H
14 R^1/R^2 = Me_2C
15a/b: R^1 = TBDPS, R^2 = H
16a/b: R^1/R^2 = Me_2C

Entry	Substrates and conditions[a]	Yield (ratio)[b]
1	**13**, Cu_2O 20 mol%, toluene	72% (1 : 10)
2	**13**, Ag_2O 20 mol%, toluene	70% (1 : 7.0)
3	**13**, *i*Pr_2NEt 10 mol%, DCM	< 5% (ND)[c]
4	**13**, Zn(OTf)2 10 mol%, *i*Pr_2NEt 10 mol%, DCM	< 5% (ND)[c]
5	**13**, Cu(OTf)2 10 mol%, *i*Pr_2NEt 10 mol%, DCM	75% (1 : 12)
6	**13**, Sc(OTf)3 10 mol%, *i*Pr_2NEt 10 mol%, DCM	<5% (ND)[c]
7	**13**, Cu(*t*ButSal)$_2$ 10 mol%, *i*Pr_2NEt 10 mol%, DCM	80% (1 : 10)
8	**13**, LiBF4 100 mol%, *i*Pr_2NEt 10 mol%, DCM	35% (1 : 4.3)
9	**13**, LiBF4 100 mol%, *i*Pr_2NEt 10 mol%, DCE	61% (1 : 2.4)
10	**13**, LiBF4 100 mol%, *i*Pr_2NEt 10 mol%, DME	32% (1 : 5.6)
11	**13**, LiBF4 100 mol%, *i*Pr_2NEt 10 mol%, THF	<5% (ND)[c]
12	**13**, $LiBF_4$ 100 mol%, *i*Pr_2NEt 10 mol%, dioxane	<5% (ND)[c]
13	**13**, LiBF4 100 mol%, *i*Pr_2NEt 10 mol%, MeCN	65% (1 : 3.4)
14	**14**, Cu_2O 20 mol%, toluene	80% (1 : 1.8)
15	**14**, Cu_2O 20 mol%, DCE	78% (1 : 2.0)
16	**14**, Cu_2O 20 mol%, THF	75% (1 : 1.8)
17	**14**, Cu_2O 20 mol%, MeCN	85% (1 : 2.3)
18	**14**, Cu(tBuSal)$_2$ 10 mol%, *i*Pr_2NEt 10 mol%, DCM	70% (1 : 1.5)
19	**14**, $LiBF_4$ 100 mol%, *i*Pr_2NEt 10 mol%, DCE	72% (2.0 : 1)
20	**14**, $LiBF_4$ 100 mol%, *i*Pr_2NEt 10 mol%, DCE-DMF	55% (5.0 : 1)
21	**14**, $LiBF_4$ 120 mol%, *i*Pr_2NEt 10 mol%, DCE-DMF	60% (4.8 : 1)
22	**14**, $LiBF_4$ 200 mol%, *i*Pr_2NEt 10 mol%, DCE-DMF	65% (4.7 : 1)
23	**14**, LiOTf 200 mol%, *i*Pr_2NEt 10 mol%, DCE-DMF	68% (5.1 : 1)
24	**14**, $LiNTf_2$ 200 mol%, *i*Pr_2NEt 10 mol%, DCE-DMF	64% (4.8 : 1)

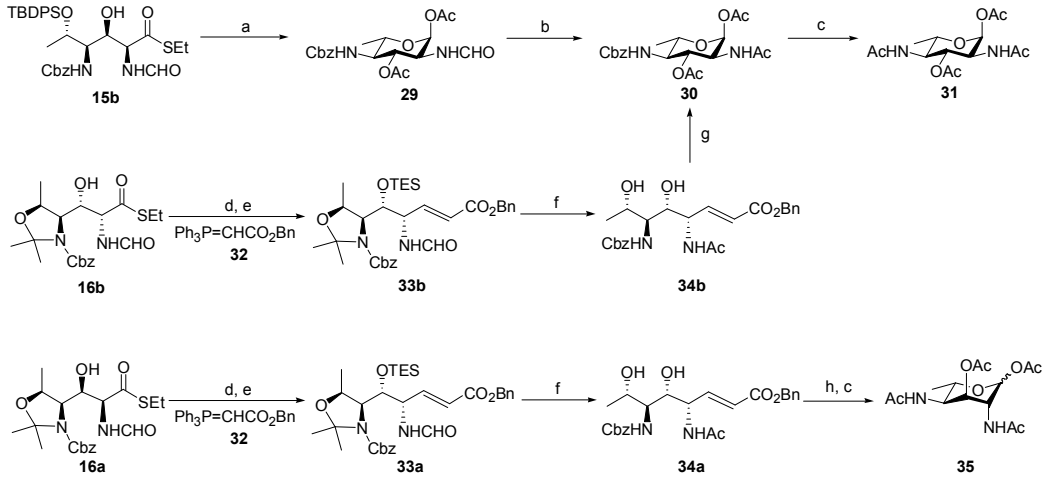
a) All the condition screening experiments were done using 0.10 mmol aldehyde and 0.12 mmol isonitrile at r.t. for 2 h. The crude mixture was concentrated, and the residue was dissolved in THF and water (2 : 1 *v*/*v*) and refluxed overnight. In most cases, a full conversion of the aldehyde and formation of the thioester product were achieved. b) The ratio was determined by ^{1}H NMR of the crude product, based on the integration of the peaks of the formyl proton. c) Trace amount of the thioester product was formed, and the ratio was not determined.

SCHEME 2. *De novo* synthesis of the glycosyl Pse donors via two chain elongations.



Reagents and conditions: a) (i) $SOCl_2$, MeOH, reflux; (ii) AcCl, Et_3N, DCM, 79% over 2 steps. b) (i) $SOCl_2$; (ii) 10% HCl (aq), reflux. c) CbzCl, Na_2CO_3, H_2O. d) MeI, $KHCO_3$, DMF, 77% over 4 steps. e) For **11**: TBDPSCl, imidazole, DMF, 24 h, 95%. f) For **12**: 2,2-dimethoxypropane, BF_3OEt_2, DCM, 12 h, 95%. g) For **13**: DIBAL-H, DCM, -78 °C, 2 h, 87%. h) For **14**: (i) $NaBH_4$, $CaCl_2$, EtOH-THF, 24 h, 95% (97% BRSM). (ii) BAIB, TEMPO, DCM, 0 °C to r.t., 10 h, 88%. i) For **15**: (i) $CNCH_2COSEt$ (**8**), Cu_2O, toluene, 40 °C, 2 h; (ii) THF, H_2O, reflux, 12 h, 72%, dr 1 : 10, over 2 steps. j) For **16**: (i) $CNCH_2COSEt$ (**8**), LiOTf, *i*Pr_2NEt, DCE-DMF, r.t., 3 h; (ii) THF-H_2O, reflux, 10 h, 67%, dr 5.1 : 1, over 2 steps. k) Et_3SiH, Pd/C, THF, 3 h. l) **18**, In powder, NH_4Cl, EtOH, 2 h, 82%. m) Dess-Martin reagent, DCM, 0 °C, 2 h, 98%. n) TBAF, HOAc, THF, 1 h. o) $NaBH(OAc)_3$, HOAc, MeCN, -40 to -20 °C, 10 h, 92% over 2 steps. p) HOAc, H_2O, 50 °C, 20 h, 72% (2 rounds reaction). q) 3% HCl aq in MeOH, 0 °C to r.t., 8 h. r) TrocCl, 0.5 M Na_2CO_3, MeCN, 2 h, 69%. s) O_3, DCM, -78 °C, 0.5 h; then Me_2S, 91%. t) Ac_2O, pyridine, DMAP, 89%. u) TolSH, BF_3OEt_2, DCM, 16 h, 80%. v) HCl aq, acetone-H_2O, reflux, 16 h, 95%. w) For **27**: (i) TBAF, HOAc, THF, r.t.12 h; (ii) Ac_2O, pyridine, 87% over 2 steps. x) For **28**: Chloroacetyl anhydride, NMM, DMAP, DCM, 90%.

SCHEME 3. Elucidation of the configurations of isonitrile adducts via derivatization.



Reagents and conditions: a) (i) Pd/C, Et_3SiH, DCM, 2 h; (ii) TBAF, THF, 12 h; (iii) Ac_2O, Py, 2 h, 42% over 3 steps. b) (i) 3% HCl (aq) in MeOH, 0 °C to r.t., 8 h; (ii) Ac_2O, Py, DMAP, 1 h, 62% over 2 steps. c) (i) $Pd(OH)_2/C$, H_2, MeOH, 2 h; (ii) Ac_2O, Py, DMAP, 75% for **31**, 69% for **35**, over 2 steps. d) Pd/C, Et_3SiH, THF, 2h, then 1 M HCl (aq), DCM, 45 min. e) $Ph_3P=CHCO_2Bn$ (**32**), DCM, 45 min, 84% for **33a**, 27% for **33b**, over 2 steps. f) (i) TFA, H_2O, 0 °C, 0.5 h; (ii) 3% HCl (aq) in MeOH, 0 °C to r.t., 8 h; (iii) Ac_2O, Na_2CO_3 (aq), MeCN, 2 h, 59% for **34a**, 63% for **34b**, over 3 steps. g) (i) O_3, DCM, -78 °C, 0.5 h; (ii) Ac_2O, Py, DMAP, 1 h, 67% over 2 steps. h) (i) O_3, DCM, -78 °C, 0.5 h; (ii) Ac_2O, *N*-methylmorpholine, DMAP, DCM, 93% over 2 steps.

The application of glycine thioester isonitrile **8** was pivotal for this success, while the corresponding glycine *O*-ester isonitrile gave the undesired diastereomer as the major product even under the optimized condition for **8** (see Supporting Information). The thioester group in **8** not only ensured the high reactivity (likely through lowering the pKa of the methylene

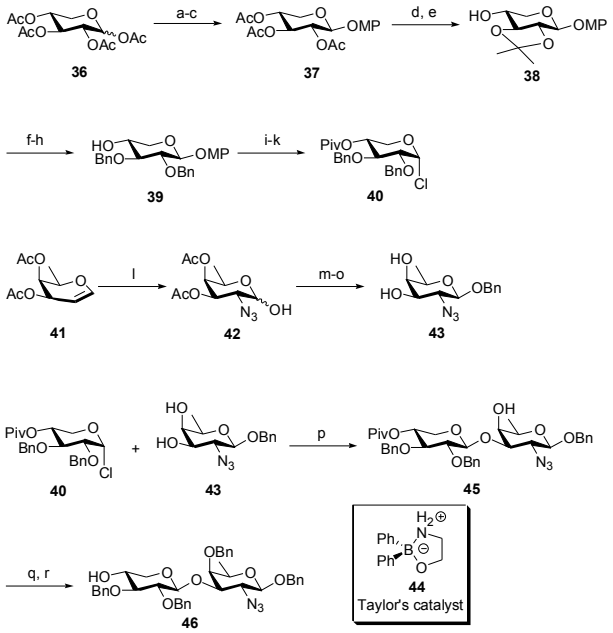
proton) and selectivity in this chain elongation step (from **14** to **16**), but also served as an excellent aldehyde group surrogate to afford **17** for the subsequent chain elongation (*vide infra*), while all attempts to convert the corresponding *O*-ester counterpart into **17** failed. The LiOTf, thanks to better solubility, gave slightly higher diastereoselectivity (5.1 : 1 ratio) and highly reproducible 68% yield (entry 23). Therefore, it was chosen as the optimized condition for scale-up synthesis to provide **16a** in 10 g scale.

In the second chain elongation, the thioester **16a** was smoothly transformed into the aldehyde **17** via the Fukuyama reduction.[29] Partial TES installation on the 3-hydroxyl group of **17** (the 6-hydroxyl group of Barbier adduct **19)** was observed when the reduction was carried out in DCM. Surprisingly, when THF was used instead, complete TES protection was observed. To the best of our knowledge, though THF was seldom used in the Fukuyama reduction,[30] the accompanying *in situ* TES protection has never been reported before. More importantly, this outcome provided us an opportunity for the effective configuration adjustment of the Barbier adduct (*vide infra*). In the indium mediated Barbier reaction with **18**, the adduct **19** was formed as a 3 : 1 ~ 5 : 1 mixture of diastereomers. The two diastereomers could be separated, but unfortunately the undesired *syn* adduct with *R* configuration was the major product.[31, 32] All the attempts of the direct chirality inversion via the Mitsunobu reaction failed. At last, the configuration was then successfully adjusted to *S* via the Dess-Martin oxidation/desilylation/1,3-induced diastereoselective reduction process to provide **21** in a high overall yield (90% for 3 steps). After deprotection of the acetonide group under the mild acidic condition and hydrolysis of the formamide by 3% HCl (aq) in MeOH,[33] the liberated amino group was reprotected by a Troc group to give **23**. Thus, the 1,3-*anti*-diamino skeleton with correct configurations and orthogonal protecting groups was constructed. The isopropyl ester was critical for the successful acidolysis of the formamide in **22**, while the less bulky methyl and ethyl esters led to the cyclization product γ-butyrolactone.

After cleavage of the alkene of **23** by ozonolysis and subsequent acetylation, the cyclic product **24** with the Pse full skeleton was formed, and the two anomers were characterized by 2D NMR to further confirm the configuration of the newly formed chiral center. The Pse thioglycoside donor **25** was synthesized as the single α anomer via the BF3·OEt2 mediated thioglycosylation ($J_{C1\text{-}H3a}$ = 0 Hz). This synthetic route was efficient (16 steps from **10** in 11% yield) for scalable preparation of **25**. The donor **27** with *O*4,*N*5-cyclic carbamate structure was synthesized via acidic acetyl group removal and TBAF-mediated Troc deprotection/cyclization.[34] The donor **28** with chloroacetyl (ClAc) groups installed on *O*4 and *O*8 was also synthesized from **26**.

2.3 Synthesis of the β-Xyl-(1→3)-FucNAc disaccharide acceptor via the Taylor glycosylation. Our preliminary glycosylation studies of Pse donors and the OMP-2,3-dibenzolyated xyloside acceptor showed that such a disarmed acceptor had very low reactivity towards the Pse glycosylation leading to very low yield of the produced disaccharide. Thus, we synthesized an armed disaccharide **46** as the glycosyl acceptor.

SCHEME 4. Synthesis of disaccharide acceptor 46 via the Taylor β-selective glycosylation.

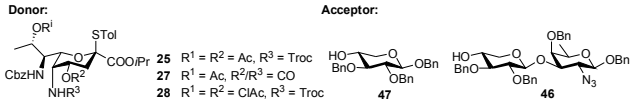


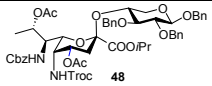
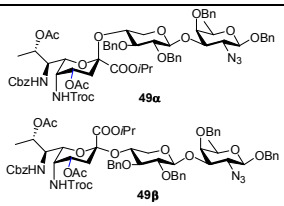
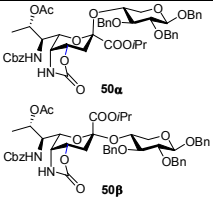
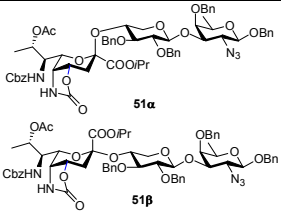
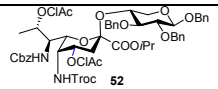
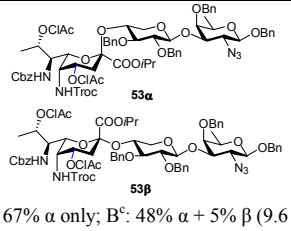
Reagents and conditions: a) Ethylenediamine, HOAc, THF, 70%. b) CCl_3CN, DBU, DCM, 79%. c) 4-Methoxyphenol, BF_3OEt_2, 4 Å molecular sieves, DCM, 0 °C to r.t., 73%. d) MeONa, MeOH. e) 2-Methoxypropene, TFA, DMF, 50 °C, 71% over 2 steps. f) PMBCl, NaH, DMF, then Camphorsulfonic acid, DCM-MeOH, 89%. g) BnBr, NaH, DMF, 94%. h) 10% TFA in DCM, 88%. i) PivCl, Py, DMAP, 60 °C, 70%. j) CAN, MeCN, H_2O, 0 °C, 70%. k) Oxalyl chloride, DCM, DMF, 65%. l) CAN, NaN_3, MeCN, -20 °C, then TolSH, *i*PrNEt$_2$, MeCN, 57%. m) CCl_3CN, DBU, DCM, 73%. n) BnOH, BF_3OEt_2, 4 Å molecular sieves, DCM, -78 °C, 73%. o) MeONa, MeOH, 93%. p) Taylor's catalyst **44**, Ag_2O, MeCN, 16 h, 83%. q) BnBr, NaH, 4 Å molecular sieves, DMF, 0 °C, 4 h, 89%. r) MeONa, MeOH, 4 h, 98%.

To this end, the D-xylosyl chloride donor **40** was synthesized from D-xylose tetraacetate **36** in 11 steps (Scheme 4). The *O*1 of xylose was temporarily protected by 4-methoxyphenyl (MP) group to afford **37**. Next, the *O*2 and *O*3 were selectively blocked by an acetonide group following the known procedure.[35] After installation of the PMB group on *O*4 and benzyl group on *O*2/*O*3, the anomeric hydroxyl group was released by ammonium cerium(IV) nitrate (CAN) oxidation and was transformed to the xylosyl chloride **40** via oxalyl chloride mediated chlorination. The D-fucosamine acceptor **43** was prepared from D-fucal **41** in 4 steps. The azide group was installed at *C*2 equatorially via oxidation,[36] and the *O*1-benzyl group was stereoselectively installed via the acid-base catalyzed β-glycosylation developed by Schmidt et al.[37] The donor **40** lacked of neighboring group participation to ensure β-glycosylation with **43**. This problem was finally solved by using the Taylor glycosylation.[38] To our delight, the 2-aminoethyl diphenylborinate **44** catalyzed the glycosylation regioselectively and stereoselectively to give disaccharide **45** with absolute β(1, 3) linkage in 83% yield. After subsequent benzylation and Piv removal, the acceptor **46** was obtained.

2.4 Glycosylation studies of the Pse glycosyl donors. With the Pse glycosyl donors (**25**, **27**, **28**) in hand, we started to investigate the glycosylation. Two acceptors, xyloside **47**[39] and disaccharide **46** were tested, and the results are shown in Table 2.

TABLE 2. Investigation on the glycosylation of Pse donors.



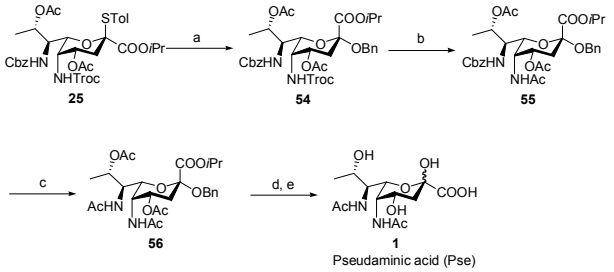
Donor/Acceptor	Products (yield and ratio)[a]
25/**47**	 **48** A[b]: 68% α only; B[c]: 35% α only
25/**46**	 **49α** **49β** A[b]: 67% α only; B[c]: 48% α + 5% β (9.6 : 1)
27/**47**	 **50α** **50β** A[b]: 20% α + 24% β (1 : 1.2); B[c]: 29% α + 12% β (2.4 : 1)
27/**46**	 **51α** **51β** A[b]: 14% α + 15% β (1 : 1.1); B[c]: 18% α + 11% β (1.6 : 1)
28/**47**	 **52** A[b]: 67% α only; B[c]: 32% α only
28/**46**	 **53α** **53β** A[b]: 67% α only; B[c]: 48% α + 5% β (9.6 : 1)

a) The ratios were calculated base on the isolated yields of the anomers. b) Condition A: donor 1.0 equiv, acceptor 2.0 equiv, TolSCl 3.0 equiv, AgOTf 4.0 equiv, AW-300 molecular sieves, -78 °C. c) Condition B: donor 1.0 equiv, acceptor 2.0 equiv, TolSCl 3.0 equiv, AgOTf 4.0 equiv, AW-300 dmolecular sieves, DCM/MeCN 3/1 *v*/*v*, -78 °C.

The toluenesulfenyl chloride (TolSCl)/AgOTf system[40] was chosen to activate the thioglycoside donors, while the NIS/TMSOTf or NIS/TfOH system commonly used in the sialylation failed to activate the Pse donor at -78 °C in our hands. The glycosylations were conducted at -78 °C, while reactions at -40 °C gave inferior yields with significant formation of the glycal. For the reaction of **25** with **47**, the disaccharide **48** was obtained as the single α anomer in both two solvent systems (DCM and DCM/MeCN). The configuration of the α-linkage was elucidated by HMBC spectrum, in which no correlation between C1 and H3a was observed ($J_{C1\text{-}H3a}$ = 0 Hz). The concentration of the reaction was found to be critical, as the yield of **48** dropped significantly to <5% accompanied by the formation of the glycal, when the concentration of **25** decreased from 50 mM to 25 mM (the concentration of **47** decreased correspondingly from 100 mM to 50 mM). In the glycosylation of more challenging disaccharide **46**, only **49α** was formed in 67% yield in the DCM system, while the mixture of **49α** and **49β** were formed in 9.6 : 1 ratio in the DCM/MeCN system. The configurations of both anomers were elucidated by the HMBC spectra ($J_{C1\text{-}H3a}$ = 0 Hz for **49α** and $J_{C1\text{-}H3a}$ = 6.7 Hz for **49β**). When donor **27** containing a cyclic carbamate was tested, a significantly different behavior was observed. In the glycosylation of both **47** and **46**, the mixtures of anomers **50α**/**β** and **51α**/**β** were obtained respectively, albeit in a low ratio and low yield, affected by the formation of a significant amount of the glycal. The addition of MeCN decreased the amount of β anomers (carboxyl groups orient axially), which was in contrast with the sialylation chemistry.[41] This illustrated the important effect of the *C*5 amido group on both reactivity and selectivity of the Pse donor.[42] The configuration of **50α** and **51α** were elucidated through derivatization of **48** and **49α**, respectively. When donor **28** with chloroacetyl (ClAc) group was tested, similar results to donor **25** were obtained in DCM system, while lower yield of **52α** and **53α**/**β** were obtained in DCM/MeCN system respectively ($J_{C1\text{-}H3a}$ = 0 Hz for **52α**/**53α** and $J_{C1\text{-}H3a}$ = 6.6 Hz for **53β**).

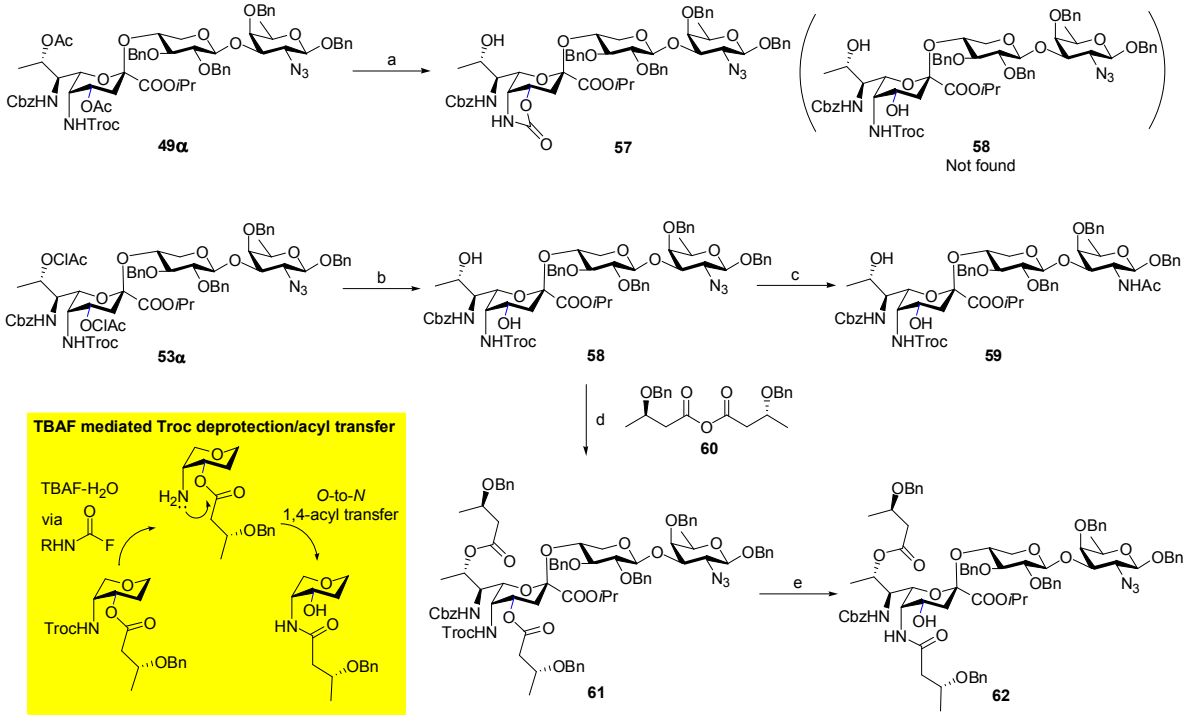
2.5 Total synthesis of Pse 1. After glycosylation of **25** with benzyl alcohol (4.0 equiv), compound **54** was obtained as single β anomer ($J_{C1\text{-}H3a}$ = 6.6 Hz) in 94% yield, and was further transformed into **1** through sequential manipulation of Troc and Cbz groups into acetyl groups, saponification of *O*-acetate and isopropyl ester, and hydrogenolysis of the benzyl group (Scheme 5). The final product **1** shows ^{1}H and ^{13}C NMR spectra identical to the published results,[23a] which unambiguously confirms the correct construction of all the three new chiral centers on the Pse carbon chain along our synthetic route.

SCHEME 5. Total synthesis of pseudaminic acid 1.



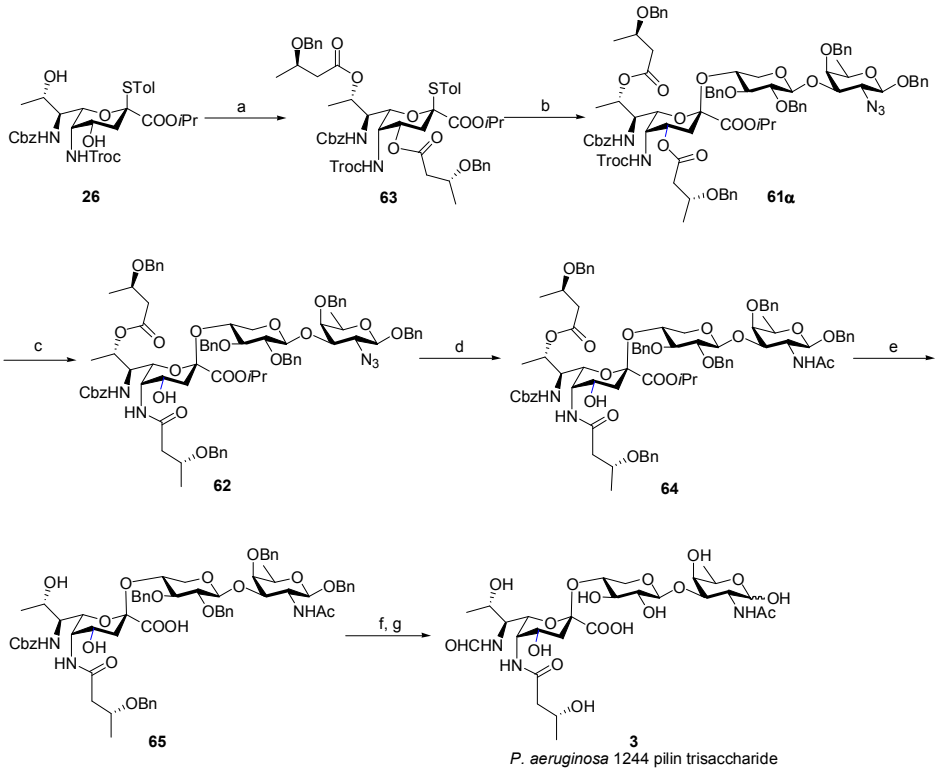
Reagents and conditions: a) BnOH, TolSCl, AgOTf, DCM, AW-300 MS, -78 °C, 2 h, 94%, only β. b) Zn powder, HOAc, Ac_2O, 50 °C, 74%. c) Pd/C, H_2, NH_4OAc, MeOH-DCM; then Ac_2O, pyridine, 93%. d) LiOH, THF-H_2O. e) Pd/C, H_2, MeOH-H_2O, 90% over 2 steps.

SCHEME 6. Attempts for side chain manipulations on the trisaccharide structures.



Reagents and conditions: a) 8 M $MeNH_2$ in EtOH, THF, 24 h, 57%. b) DABCO 15 eq., EtOH, 55 ^oC, 2 h, 76%. c) PMe_3, THF, HOAc; then H_2O; then DABCO, Ac_2O, MeOH, 56%. d) 3-Benzyloxy butanoic anhydride **60**, pyridine, DMAP, 77%. e) TBAF, HOAc, THF, 40 ^oC, 48 h, 77%.

SCHEME 7. The final route towards the total synthesis *P. aeruginosa* 1244 pilin trisaccharide 3.



Reagents and conditions: a) (*R*)-3-benzyloxybutanoic anhydride **60**, pyridine, DMAP, 12 h, 77%. b) Condition A: **46**, TolSCl, AgOTf, DCM, AW-300 MS, -78 ^oC, 2 h, 66%, only α. Condition B: **46**, TolSCl, AgOTf, MeCN-DCM, AW-300 MS, -78 ^oC, 2 h, 49% for α, 9% for β. c) TBAF, HOAc, THF, 40 ^oC, 48 h, 77% (84% BRSM). d) $NiCl_2$-$6H_2O$, $NaBH_4$, MeOH, 0 ^oC, 1 h, then Ac_2O, 0 ^oC, 1 h, 76%. e) LiOH, THF, H_2O, 99%. f) H_2, Pd/C, HOAc-H_2O, 48 h. g) Formic anhydride (solution in Et_2O), Et_3N, MeOH, -20 ^oC, 5 h, 75% over 2 steps.

2.6 The synthetic routes toward trisaccharide 3 and endgame. With the desired trisaccharide **49α** in hand, we next started to install the side chains (Scheme 6). Based on the orthogonal deprotection conditions for Troc, azide, and

Cbz groups, we decided to install the (*R*)-3-hydroxybutyryl group, acetyl group, and formyl group sequentially.For installation of the (*R*)-3-hydroxybutyryl group onto *N*5 of Pse, to avoid the undesired *O*4 to *N*5 acyl transfer, the *O*-acetyl groups must be removed before the deprotection of Troc. However, under all the tested conditions (including MeONa or K_2CO_3 in MeOH or mixed solvents as well as other weaker base systems), only the *O*4,*N*5-carbamate product **57** was isolated in variable yields, without formation of the desired product **58**. This result could be attributed to the *cis* relationship of the 4- and 5-substitutions, which rendered the OH and the carbonyl carbon of Troc group in appropriate proximity for an intramolecular cyclization. The difficulties that we confronted for further activation and cleavage of the cyclic carbamate led us to search for other protecting groups which could be removed under the milder basic conditions.

We next chose to start the side chain manipulation from trisaccharide **53α** with chloroacetyl (ClAc) protections. To our delight, upon treatment with DABCO in EtOH at 55 °C for 2 h,[43] both two ClAc groups were removed smoothly with the Troc group intact, and the diol product **58** was isolated in 76% yield, with the starting material and partially deprotected products recovered. However, selective reduction of the azide group on the fucosamine unit in the presence of Troc group was difficult. Through the PMe_3-mediated Staudinger reduction and subsequent acetylation, the product **59** was obtained in only 56% yield. The addition of HOAc during the Staudinger reduction was critical for the success, which suppressed the formation of cyclic carbamate from Troc group mediated by the highly basic iminophosphorane intermediate. However, this result was far from satisfactory.

At this stage, we designed a new strategy for side chain installation on *N*5, based on TBAF-mediated Troc deprotection and *O*4 to *N*5 acyl transfer. As illustrated in Scheme 6, the 3-benzyloxy butyrate group was installed onto both *O*4 and *O*8 of **58** simultaneously using the corresponding anhydride **60** in 77% yield. The intermediate **61** was then treated with TBAF and HOAc in THF. Gratifyingly, the desired *N*5 amide product **62** was obtained in 77% yield. The reaction needed 48 h at 40 °C to achieve good conversion with minimized side reactions, while higher temperature gave faster conversion but lower yield. During this transformation, the putative carbamoyl fluoride intermediate proposed by Coudert et al.[34b] was hydrolyzed *in situ* by the trace amount of water, and the released amino group underwent the *O*-to-*N* 1,4-acyl transfer. Encouraged by this success of such a TBAF-mediated Troc deprotection/acyl transfer strategy, we designed a new synthetic route towards the final *Pseudomonas aeruginosa* 1244 pilin trisaccharide **3** to further shorten the reaction sequence and improve the synthetic efficiency.

The final route towards the pilin glycan trisaccharide is shown in Scheme 7. To save the ClAc protection/deprotection steps, the desired (*R*)-3-benzyloxybutyrate side chain was directly installed onto the *O*4 and *O*8 of diol **26** to form Pse glycosyl donor **63**. The glycosylation between **63** and **46** proceeded smoothly in DCM to provide the desired **61α** anomer in 66% yield, while a mixture of **61α** and **61β** anomers was obtained in lower yield when the glycosylation was performed in DCM-MeCN mixture ($J_{H3a\text{-}C1}$ = 0 Hz for **61α** and $J_{H3a\text{-}C1}$ = 6.6 Hz for **61β**). After TBAF-mediated Troc deprotection of **61α** and subsequent acyl transfer, the rearranged product **62** was obtained in 77% yield. Subsequently, the azide group was reduced by the nickel boride reagent, followed by *in situ* acetylation by Ac_2O to give **64** in 76% yield. After mild saponification, the resultant acid **65** was treated with H_2 and Pd/C in HOAc-H_2O to remove the Cbz group and five benzyl groups, and the desired formyl group was next installed onto the released *N*7 by formic anhydride[44] (freshly prepared from formic acid and DCC in Et_2O at -40 °C) at -20 °C. The final product *Pseudomonas aeruginosa* 1244 pilin trisaccharide **3** was obtained in 75% yield after C18 silica gel column chromatography separation and lyophilization. The structure of **3** was characterized by ^1H, ^{13}C, and 2D NMR, as well as high resolution ESI-MS.

CONCLUSION

In summary, we developed a facile and scalable *de novo* synthesis of pseudaminic acid (Pse) and its functionalized derivatives from L-threonine. The key features in the synthesis include the diastereoselective glycine thioester isonitrile-based aldol-type reaction to create the 1,3-*anti*-diamino skeleton with orthogonal functionalities, the Fukuyama thioester reduction and the indium-mediated Barbier-typed allylation. In particular, the finding that the Fukuyama thioester reduction in THF accompanied *in situ* hydroxyl TES protection made it possible to effectively adjust the configuration of the hydroxyl group generated after the Barbier-typed allylation via the Dess-Martin oxidation/desilylation/1,3-induced diastereoselective reduction. These strategies and findings are expected to find broad applications in the synthesis of diamino sugars widely existing in bacterial glycans.[45]

Furthermore, we fully investigated the glycosylation of Pse donors and identified the structural determinants for its glycosylation diastereoselectivity. Thus, we completed the first total synthesis of *Pseudomonas aeruginosa* 1244 pilin trisaccharide (α-5NβOHC$_4$7NFmPse-(2→4)-β-Xyl-(1→3)-FucNAc), which enabled us to confirm the configuration of the Pse-Xyl α-glycosidic linkage via HMBC analysis of the synthetic intermediates, as the original report determined it only based on the 1D ^1H NMR with empirical rules due to the lack of sufficient amount of the glycan. The free lactol at the reducing end of the trisaccharide provides us the possibility for installing thiol,[46] azide[47] or bifunctional linker[48] moieties via readily available methodologies, which could be used in the synthesis of glycoconjugates (e.g. neoglycopeptides). This ready access to pseudaminic acid and its glycoconjugate will open up unexplored opportunities to study the biological function and significance of bacterial Pse glycosylation in pathogenesis and develop novel therapeutic intervention.

ASSOCIATED CONTENT

Supporting Information.
Experimental procedures, characterization data of synthetic compounds, and copies of 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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