Tetrahedron Letters 52 (2011) 418–421

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of pseudaminic acid, a unique nonulopyranoside derived from pathogenic bacteria through 6-deoxy-AltdiNAc

Yong Joo Lee [a], Akemi Kubota [a], Akihiro Ishiwata [a], Yukishige Ito [a,b,*]

[a] RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[b] ERATO Ito Glycotrilogy Project, JST, Wako, Saitama 351-0198, Japan

ARTICLE INFO

Article history:
Received 21 October 2010
Revised 10 November 2010
Accepted 15 November 2010
Available online 18 November 2010

Keywords:
Pseudaminic acid
2,4-Diacetamido-2,4,6-trideoxy-L-altropyranose
In-mediated allylation

ABSTRACT

Chemical synthesis of pseudaminic acid is described. Starting from *N*-acetylglucosamine, deoxygenation and deoxyamination with stereo-inversion afforded 6-deoxy-AltdiNAc, which is the key intermediate for the biosynthesis of pseudaminic acid. Subsequently, the elongation reaction via In-mediated allylation of 6-deoxy-AltdiNAc with bromomethacrylate ester derivative followed by ozonolysis and hydrolysis gave the desired pseudaminic acid. Furthermore, we demonstrated glycosylation with dibenzyl phosphite derivative of pseudaminic acid as the glycosyl donor to afford disaccharide.

In contrary to the long standing belief, studies in recent years have revealed the presence of O- as well as N-glycosylations of proteins in various prokaryotes.[1,2] O-Linked glycosylation by pseudaminic acid **1** (Pse) is particularly intriguing. In flagellin glycoproteins derived from several pathogenic bacteria such as *Campylobacter jejuni* and *Helicobacter pylori*,[2,3] Pse is directly connected to their Ser/Thr residues and is known to be important for bacteria's motility and invasion to host cells.[2] In addition, Pse has been identified as a component of larger glycans. For instance, trisaccharide repeating unit of O-antigen polysaccharide from *Escherichia coli* O136[4] contains Pse and *Pseudomonas aeruginosa* 1244 trisaccharide was revealed to have modified pseudaminic acid.[5]

Although its similarity to sialic acid (Neu5Ac), which exists in various glycoconjugates of eukaryotic glycoproteins and glycolipids, is obvious, Pse has structural features clearly distinct from Neu5Ac (Fig. 1). Firstly, stereochemical configurations are opposite at *C*-5, -7, and -8 between them. Unlike Neu5Ac, Pse is an L-sugar and its equatorial glycoside, a naturally occurring form, is defined as β. Furthermore, Pse has a 7,9-dideoxy structure and its *C*-7 position is substituted by an *N*-acetyl or an *N*-acetimidoyl (Am) group. On the other hand, presence of highly deoxygenated and acetamido functionalized rare sugars, such as 2,4-diacetamido-2,4,6-trideoxy-D-glucopyranose (bacillosamine, Bac),[6] 2,4-diacetamido-2,4,6-trideoxy-D-galactopyranose (6-deoxy-GaldiNAc),[7] and 2,4-diacetamido-2,4,6-trideoxy-L-altropyranose (6-deoxy-AltdiNAc)[8] had been identified in various bacteria. Among them, 6-deoxy-AltdiNAc is of particular interest as a biosynthetic precursor of Pse.[9]

In spite of its growing attention, chemistry of Pse has been rather unexplored. There has been only a single reported synthesis of Pse,[10] in which condensation of oxalacetic acid and L-allose derivative was employed as the key reaction. However, this reaction gave the desired isomer as a minor product and the yield was low (3%). In order to conduct systematic studies to clarify the function of bacterial O-linked glycosylation, more practical means to supply highly purified Pse will be required. With these circumstances in mind, our study has aimed to establish synthetic route to the Pse.[11]

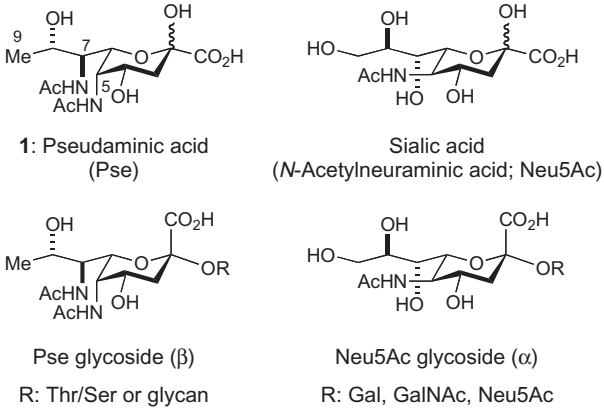
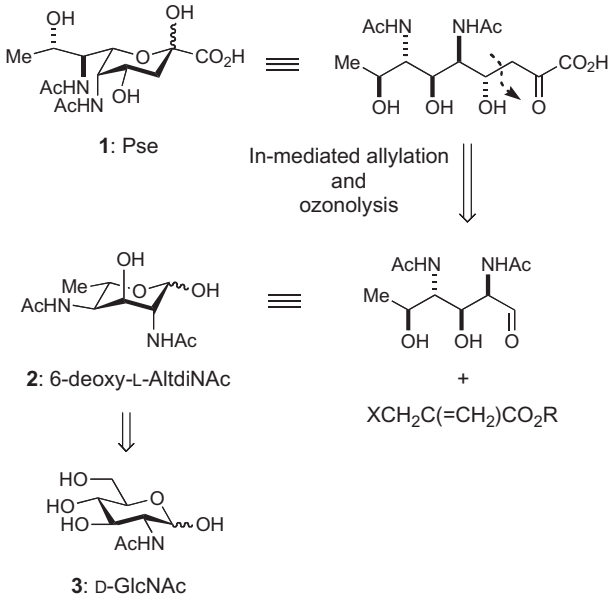


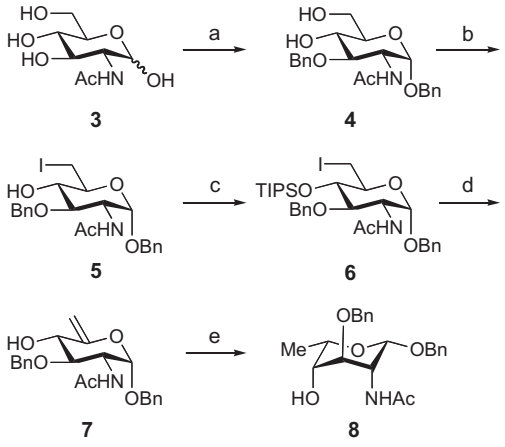
Figure 1. Pse (**1**), Neu5Ac and their glycosides.

* Corresponding author. Tel.: +81 (0)48 467 9430; fax: +81 (0)48 462 4680.
E-mail address: yukito@riken.jp (Y. Ito).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.11.078



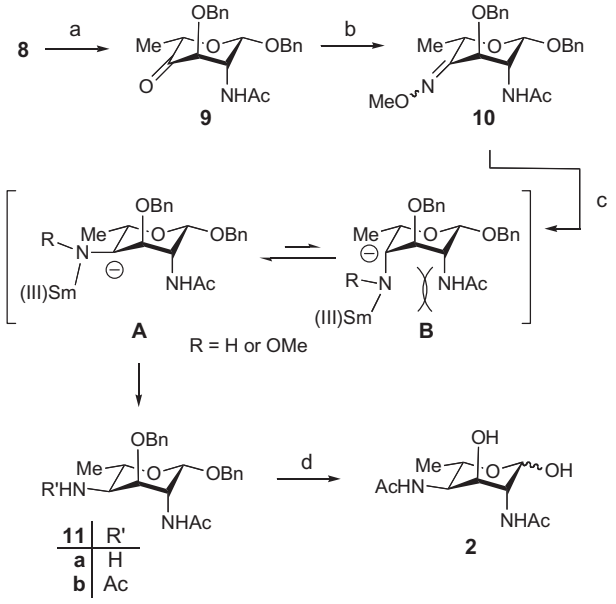
Scheme 1. Retrosynthesis of Pse (**1**).



Scheme 2. Reagents and conditions: (a) Ref.[12]; (b) I_2, PPh_3, imidazole, THF, 0 °C, 2 h, 88%; (c) TIPSOTf, 2,6-lutidine, CH_2Cl_2, 12 h, 92%; (d) *t*-BuOK, THF, 70 °C, 10 h, then TBAF, THF, 2 h, 96%; (e) H_2, $RhCl(PPh_3)_3$, benzene, EtOH, 3 h, 84%.

Outlined in Scheme 1 is our synthetic plan toward Pse, which intended to mimic its biosynthetic pathway, as it employed 6-deoxy-AltdiNAc **2** as the key intermediate.[9] To be brief, starting from *N*-acetylglucosamine **3**, deoxygenation, and deoxyamination with stereochemical inversion were expected to afford the 6-deoxy-AltdiNAc **2**. Subsequent In-mediated allylation was going to give the framework of Pse **1** (Scheme 1).

As a precursor of **2**, L-idosamine derivative **8** was prepared as depicted in Scheme 2. To begin with, *N*-acetylglucosamine **3** was converted to benzyl glycoside **4** according to the known procedure.[12] Iodination with I_2, imidazole, and Ph_3P cleanly afforded compound **5**. Subsequent β-elimination in the presence of DBU gave **7**, however, in a modest yield (45%). More practically, the compound **5** was once converted into 4-*O*-trisopropylsilyl (TIPS) ether **6**, treatment of which with *t*-BuOK caused clean formation of the olefin, and subsequent desilylation gave 5,6-anhydroglucosamine derivative **7** in 88% overall yield. Saturation of the exocyclic olefin was conducted by catalytic hydrogenation in the presence of Wilkinson's catalyst. Pleasingly, it afforded stereoselectively the

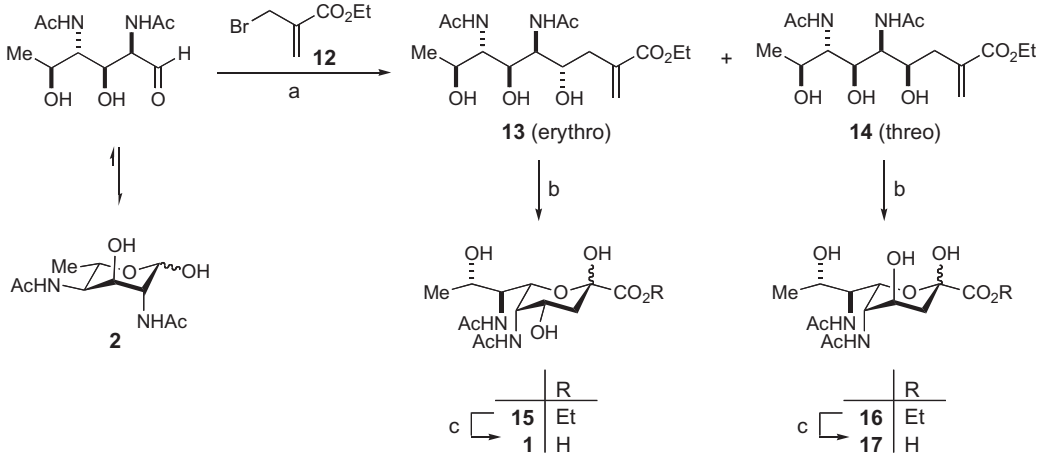


Scheme 3. Reagents and conditions: (a) Dess–Martin periodinane, $NaHCO_3$, CH_2Cl_2, 17 h, 94%; (b) $MeONH_2{\cdot}HCl$, $NaHCO_3$, MeOH, 65 °C, 17 h, 95%, major/minor = 2:1; (c) SmI_2, MeOH, THF, 12 h, then Ac_2O, pyridine, 6 h, 66%; (d) H_2, $Pd(OH)_2$, EtOH, 4 h, 94%, α/β = 2:1.

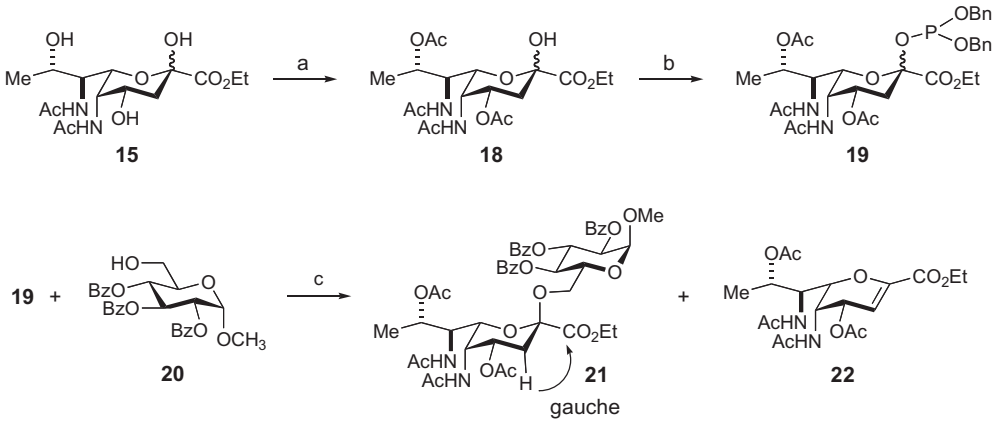
desired 2,6-dideoxy-*N*-acetyl-L-idosamine derivative **8** in 84% yield together with a minor amount (4%) of 6-deoxy-D-glucosamine type stereoisomer (Scheme 2).

Introduction of an amino-functionality to *C*-4 position of the compound **8** with stereochmeical inversion turned out to be non-trivial. Unexpectedly, S_N2-type displacements of various sulfonate esters derived from **8** with N_3 anion were not successful. As an alternative approach, reductive amination through oxime derivative was examined. Thus, oxidation of alcohol **8** with Dess–Martin periodinane gave ketone **9**, which was treated with *O*-methylhydroxylamine hydrochloride and $NaHCO_3$ to afford the oxime **10** as a 2:1 mixture due to the *E*/*Z* isomerism. Since configuration of the C=N linkage was not consequential, rigorous assignment of the stereochemistry was not made. Subsequent reduction of **10** with SmI_2[13] in THF containing MeOH followed by immediate acetylation of resultant **11a** afforded the desired 4-acetamidoaltrose derivative **11b** as a single isomer. We speculate that initially formed carbanion is under equilibration between two isomers **A** and **B**. Between them, equatorially oriented anion **B** would be less favored because of destabilization by 1,3-diaxial repulsion between steric hinderance of Sm(III)[13b] and NHAc and formation of **11a** via **A** was predominant.[14] Finally, hydrogenolysis of benzyl ether of **11b** afforded 6-deoxy-AltdiNAc **2** (Scheme 3), which corresponds to the biosynthetic intermediate of Pse.

Thus obtained **2** was subjected to chain elongation through In-mediated allylation.[15] Namely, it was reacted with bromomethacrylate ester **12** under Whitesides' conditions to give the desired erythro product **13** together with a slightly smaller amount of its *threo* isomer **14** (77%, **13**:**14** = 5:4). Although all attempts to improve the selectivity, including the use of Lewis acid, such as $La(OTf)_3$, $Ba(OTf)_3$, or $Ce(OTf)_4{\cdot}xH_2O$ in various amounts, in the presence or absence of chiral ligand,[16] failed to improve the selectivity; chromatographic separation allowed us to obtain stereochemically homogeneous **13**. Subsequent ozonolysis was accompanied by spontaneous cyclization of the 2-oxo product and cleanly afforded, after purification by a column of Iatrobeads ($CHCl_3$/MeOH/H_2O, 65:30:5), ethyl pseudaminate **15** (85%, α:β = 5:1). Finally, saponification of **15** under mildly basic condi-



Scheme 4. Reagents and conditions: (a) In, 0.1 N HCl–EtOH (1:6), 40 °C, 12 h, 77%, **13**/**14** = 5:4; (b) O_3, MeOH, −78 °C, 30 min, then 30% H_2O_2, H_2O, HCO_2H, 90 min, for **15**: 85%, α/β = 5:1; for **16**: 86%, α/β = 3:1; (c) TEA–H_2O (1:3), 0 °C, 2 h, for **1**: 96%, for **17**: 92%.



Scheme 5. Reagents and conditions: (a) Ac_2O, pyridine, 0 °C, 12 h, 73%, α/β = 8:1; (b) $Et_2NP(OBn)_2$, 1*H*-tetrazole, THF, 2 h, 94%; (c) TMSOTf, CH_3CN, 0 °C, 3 h [**21**: 28% (α:β = 10:1), **22**: 47%] or CH_2Cl_2, rt, 6 h [**21**: 35% (α only), **22**: 62%].

tions in 25% TEA–H_2O[17] gave desired pseudaminic acid **1**.[9a] Its 4-*epi*-isomer **17** was also obtained from *exo*-methylene **14** through ethyl ester **16** by the same procedure (Scheme 4).[18,19]

With synthetic Pse ester **15** in hand, our effort was extended to examine glycosylation with Pse donor. Naturally occurring Pse, so far identified, is known to exist as a β (equatorial)-glycoside (Fig. 1). Being aware of ample examples of glycosylation using Neu5Ac thioglycoside, which predominantly gave equatorial glycoside (in this case, α; see Fig. 1) in CH_3CN, our initial effort was devoted to prepare thioglycoside of Pse. However, thioglycoside formation from the peracetate derived from **15**, under the conditions established for Neu5Ac,[20] was extremely sluggish. We then turned our attention to the preparation of hemiketal **18**. Although controlled acetylation under acidic conditions[21] was not successful, treatment of **15** with 3 equiv of Ac_2O in pyridine at 0 °C gave the desired hemiketal **18** in 73% yield. Subsequent treatment with dibenzyl *N*,*N*-diethylphosphoramidite and tetrazole gave dibenzyl phosphite **19** in 94% yield,[22] which was tested as the Pse donor. In fact, glycosylation with **20** in the presence of TMSOTf[22] (0.6 equiv) in CH_3CN afforded the disaccharide **21**[23] in 28% yield. However the product mainly consisted of the α (axial)-glycoside (α:β = 10:1) and a larger amount (47%) of 2,3-dehydro derivative **22** was formed (Scheme 5). The same reaction in CH_2Cl_2 solely gave the α-glycoside in somewhat higher yield (35%) together with 62% yield of **22**. Stereochemistry of the newly generated glycosidic linkage was determined by HMBC,[23] presence of gauche correlation between proton of H-3_{ax} and carbonyl carbon of the ethyl ester being especially diagnostic. Obviously, the property Pse is markedly different from Neu5Ac, preventing selective formation of equatorial glycoside by using methods standardized in Neu5Ac chemistry.

In conclusion, we have achieved chemical synthesis of pseudaminic acid from *N*-acetylglucosamine through 6-deoxy-AltdiNAc as the key intermediate. Furthermore, the first example of glycosylation with Pse donor was presented. Stereocontrolled formation of β-Pse glycosides will be a subject of future study.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (B) (No. 22510243, A.I.) by the Ministry of Education, Culture, Sports, Science, and Technology, Foreign Postdoctoral Researcher Program (Y.J.L), and Incentive Research Grant (2009) and Fund for Seeds of Collaborative Research (2010) in RIKEN (A.I.). We thank Mrs. A. Takahashi and Mrs. S. Shirahata for technical assistance, and Dr. M. N. Amin for his effort on the unsettled phase of this work.

Supplementary data

Supplementary data (experimental procedures and ^1H and ^{13}C NMR spectra of all new compounds) associated with this article

can be found, in the online version, at doi:10.1016/j.tetlet.2010.11.078.

References and notes

1. Wacker, M.; Linton, D.; Hitchen, P. G.; Nita-Lazar, M.; Haslam, S. M.; North, S. J.; Panico, M.; Morris, H. R.; Dell, A.; Wren, B. W.; Aebi, M. *Science* **2002**, *298*, 1790–1793.
2. Thibault, P.; Logan, S. M.; Kelly, J. F.; Brisson, J.-R.; Ewing, C. P.; Trust, T. J.; Guerry, P. *J. Biol. Chem.* **2001**, *276*, 34862–34870.
3. Schirm, M.; Soo, E. C.; Aubry, A. J.; Austin, J.; Thibault, P.; Logan, S. M. *Mol. Microbiol.* **2003**, *48*, 1579–1592.
4. Staaf, M.; Weintraub, A.; Widmalm, G. *Eur. J. Biochem.* **1999**, *263*, 656–661.
5. Castric, P.; Cassels, F. J.; Carlson, R. W. *J. Biol. Chem.* **2001**, *276*, 26479–26485.
6. Young, N. M.; Brisson, J.-R.; Kelly, J.; Watson, D. C.; Tessier, L.; Lanthier, P. H.; Jarrel, H. C.; Cadotte, N.; St. Michael, F.; Aberg, E.; Szymanski, C. M. *J. Biol. Chem.* **2002**, *277*, 42530–42539.
7. (a) Medgeyes, A.; Farkas, E.; Lipták, A.; Pozsgay, V. *Tetrahedron* **1997**, *53*, 4159–4178; (b) Hermans, J. P. G.; Elie, C. J. J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1987**, *6*, 451–462.
8. (a) Liav, A.; Sharon, N. *Carbohydr. Res.* **1973**, *30*, 109–126; (b) Liu, F.; Tanner, M. E. *J. Biol. Chem.* **2006**, *281*, 20902–20909.
9. (a) Chou, W. K.; Dick, S.; Wakarchuk, W. W.; Tanner, M. E. *J. Biol. Chem.* **2005**, *280*, 35922–35928; (b) Schoenhofen, I. C.; Lunin, V. V.; Julien, J. P.; Li, Y.; Ajamian, E.; Matte, A.; Gygler, M.; Brisson, J. R.; Aubry, A.; Logan, S. M.; Bhatia, S.; Wakarchuk, W. W.; Young, N. M. *J. Biol. Chem.* **2006**, *281*, 8907–8916; (c) Schoenhofen, I. C.; McNally, D. J.; Brisson, J.-R.; Logan, S. M. *Glycobiology* **2006**, *16*, 8C–14C; (d) Schoenhofen, I. C.; McNally, D. J.; Vinogradov, E.; Whitfield, D.; Young, N. M.; Dick, S.; Wakarchuk, W. W.; Brisson, J.-R.; Logan, S. M. *J. Biol. Chem.* **2006**, *281*, 723–732; (e) Ishiyama, N.; Creuzenet, C.; Miller, W. L.; Demendi, M.; Anderson, E. M.; Harauz, G.; Lam, J. S.; Berghuis, A. M. *J. Biol. Chem.* **2006**, *281*, 24489–24495.
10. Tsvetkov, Y. E.; Shashkov, A. S.; Knirel, Y. A.; Zahringer, U. *Carbohydr. Res.* **2001**, *335*, 221–243.
11. For the chemoenzymatic azido-Pse mdification of flagella, see; Liu, F.; Aubry, A. J.; Schoenhofen, I. C.; Logan, S. M.; Tanner, M. E. *ChemBioChem* **2009**, *10*, 1317–1320.
12. Sharma, M.; Bernacki, R. J.; Paul, B.; Korytnyk, W. *Carbohydr. Res.* **1990**, *198*, 205–221.
13. (a) Molander, G. A. *Org. React.* **1994**, *46*, 211–367; (b) Hsu, D.-S.; Matsumoto, T.; Suzuki, K. *Synlett* **2005**, 801–804.
14. In ^{1}H NMR of compound **8**, observed signals with small coupling constants (J_{H1-H2} = 2, J_{H2-H3} and J_{H3-H4} = 3, J_{H4-H5} = ~0) suggested that the predominant conformation of **8** is $^{1}C_{4}$. For general disscussion about the conformation of α-D-idose; see: Grindley, T. B. In Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds., second ed.; Glycoscience, Chemistry and Chemical Biology; Springer: Berlin, 2008; Vol. 1, pp 3–55.
15. Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1993**, *58*, 7937–7938.
16. Loh, T.-P.; Zhou, J.-R. *Tetrahedron Lett.* **1999**, *40*, 9115–9118.
17. (a) Chandler, M.; Bamford, M. J.; Conroy, R.; Lamont, B.; Patel, B.; Patel, V. K.; Steeples, I. P.; Storer, R.; Weir, N. G.; Wright, M.; Williamson, C. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1173–1180; (b) Kiefel, M. J.; Wilson, J. C.; Bennett, S.; Gredley, M.; von Itzstein, M. *Bioorg. Med. Chem.* **2000**, *8*, 657–664.
18. Gao, J.; Härter, R.; Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1994**, *59*. 3741–3715.
19. Attempted saponification of **16** in aqueous ammonia gave an amide derivative of **17**. ESI-TOF HRMS: $[M+Na]^{+}$ calcd for $C_{13}H_{23}N_3NaO_7$, 356.1419, found 356.1434.
20. (a) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *187*, 35–42; (b) Hasegawa, A.; Ohki, H.; Nagahama, T.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1991**, *212*, 277–281.
21. Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *190*, 317–322.
22. For use of phosphite as leaving group of sialyl donor, see: (a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123–6126; (b) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8748–8750.
23. NMR data of compound **21α**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (t, *J* = 7.3 Hz, 3H, OCH_2CH_3), 1.25 (d, *J* = 6.9 Hz, 3H, H-9), 1.81 (t, *J* = 13.1 Hz, 1H, H-3a), 1.96 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.99 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.16 (dd, *J* = 13.1, 5.0 Hz, 1H, H-3e), 3.45 (s, 3H, OCH_3), 3.71 (d, *J* = 3.2 Hz, 2H, H-6^{Glc}), 3.82–3.90 (m, 1H, $OCHHCH_3$), 3.98–4.04 (m, 1H, $OCHHCH_3$), 4.07 (dd, *J* = 10.5, 1.4 Hz, 1H, H-6), 4.24 (dt, *J* = 10.6, 3.2 Hz, 1H, H-5^{Glc}), 4.55–4.61 (m, 2H, H-5, H-7), 5.14–5.26 (m, 4H, H-4, H-8, H-1^{Glc}, H-2^{Glc}), 5.48 (d, *J* = 11.0 Hz, 1H, NH), 5.72 (t, *J* = 10.1 Hz, 1H, H-4^{Glc}), 5.87 (d, *J* = 9.2 Hz, 1H, NH), 6.12 (t, *J* = 10.1 Hz, 1H, H-3^{Glc}), 7.25–7.56 (m, 9H, Ar*H*), 7.90–7.99 (m, 6H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.6, 21.1, 21.4, 23.4, 23.5, 32.2, 45.5, 50.5, 55.9, 62.1, 62.3, 67.2, 68.0, 68.8, 70.2, 70.3, 70.7, 72.4, 97.3, 98.8, 128.4, 128.6, 129.1, 129.3, 129.9, 130.1, 133.2, 133.4, 133.5, 164.9, 165.9, 166.0, 166.7, 170.0, 170.4, 170.6, 171.1. The crosspeak between C2 and H3a could not be observed by HMBC experiment, which suggested the formation of β-isomer. J_{H3a-H4} (~13 Hz) indicated that **21α** has chair conformation with 4-*O*-equatorial stereochemistry.