Organic LETTERS

Letter

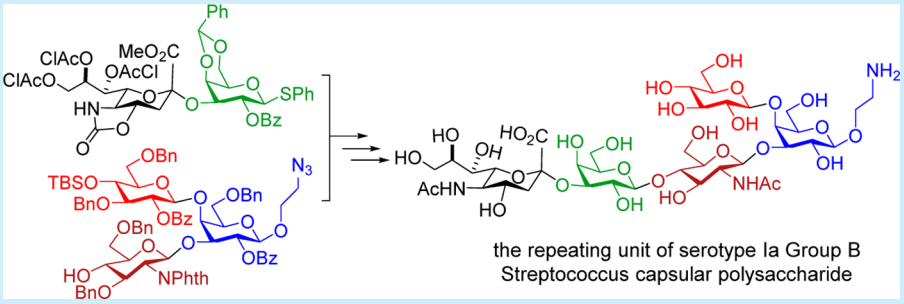
pubs.acs.org/OrgLett

Chemical Synthesis of the Repeating Unit of Type Ia Group B *Streptococcus* Capsular Polysaccharide

Prolay K. Mondal,[†] Guochao Liao,[†] Mohabul A. Mondal, and Zhongwu Guo*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202, United States

Supporting Information



ABSTRACT: The structure of the capsular polysaccharide (CPS) of serotype Ia group B *Streptococcus* (GBS) has been characterized for years, but its repeating unit, which is a challenging pentasaccharide with a branch and a difficult α-sialic acid linkage, has not been synthesized yet. In this report, an effective synthesis was developed for the serotype Ia GBS CPS repeating unit, which had a reactive functionality linked to its main-chain reducing end to enable further elaboration, such as coupling with carrier proteins. The target molecule was accomplished by a convergent [2 + 3] glycosylation strategy employing a sialo-disaccharide as donor and a branched trisaccharide as acceptor. The strategy was designed to suit the synthesis of oligomers of the repeating unit.

Streptococcus agalactiae (group B *Streptococcus*, GBS) is one of the leading causes of bacterial sepsis and meningitis among neonates.[1] Like many other pathogenic bacteria, GBS isolates also express extracellular polysaccharides with varied structures. These capsular polysaccharides (CPSs) allow for the organisms to survive within the host body by masking the antigenic determinants on the bacterial surface,[2] mimicking the host antigens,[3,4] and interfering with complement-mediated killing.[5,6] GBS has at least nine serotypes classified according to their CPS structures. Serotype Ia GBS, whose CPS structure is in Figure 1,[7] is associated with 36% of the early-onset diseases among newborn infants in the US.[8]

It has been proven that bacterial CPSs are excellent targets for carbohydrate-based antibacterial vaccine development[9] and related immunological studies.[10−12] In this regard, CPS repeating units are important, as they enable not only detailed studies of the biological functions of CPSs[13] but also development of semi and fully synthetic vaccines.[14] Thus, the repeating units of many bacterial CPSs have been synthesized. However, no synthesis of the challenging repeating unit of type Ia GBS CPS, a branched pentasaccharide with the branch linked to the galactose 3-*O*-position and a difficult α-sialic acid linkage in the side chain, has been reported. This work aimed at developing an efficient synthesis for the repeating unit of type Ia GBS CPS, as well as its derivatives suitable for further elaboration, such as preparing its oligomers, coupling to proteins, and so on, making it useful for vaccine development and other studies.

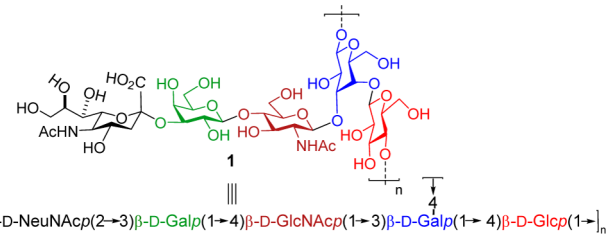


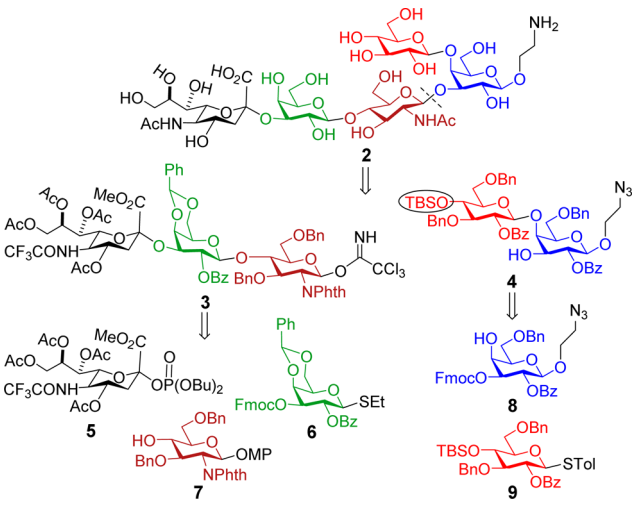
Figure 1. Structure of serotype Ia GBS CPS.

Our synthetic target **2** (Scheme 1) was the free repeating unit with an amino group at its reducing end. Our initial synthetic design intended to take advantage of the well-recognized higher reactivity of the galactose 3-hydroxyl group as compared to that of its 4-hydroxyl group[15] to accomplish a late-stage [3 + 2] glycosylation (Scheme 1). We envisioned using trichloroacetimidate **3**[16,17] as a donor and disaccharide **4** as an acceptor for the final glycosylation. In **3**, the glucosamine 2-*N*-position was protected with a phthalyl (Phth) group to guarantee β-glycosylation due to neighboring group participation. In **4**, the glucose 4-*O*-position, where the CPS grows (Figure 1), was protected with a distinctive *tert*-butyldimentylsilyl (TBS) group to provide flexibility for further elaboration, e.g., sugar chain elongation. Compound **3** would be assembled from monosaccharides **5**,[18] **6**, and **7**.[19] The 5-*N*-position in **5** and the 2-*O*-

Received: December 19, 2014

DOI: 10.1021/ol5036563
Org. Lett. XXXX, XXX, XXX−XXX

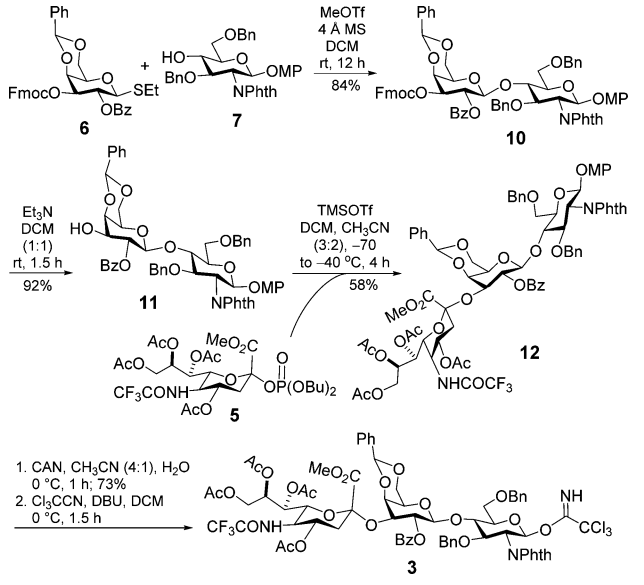
Scheme 1. Retrosynthetic Plan for the Repeating Unit of Type Ia GBS CPS 2 Based on a [3 + 2] Glycosylation Strategy



position in **6** were protected with a trifluoroacetyl and benzoyl (Bz) group, respectively, to facilitate stereoselective α-sialylation[20] and β-glucosylation, and the anomeric position in **7** was protected with a *p*-methoxyphenyl (MP) group that can be removed with mild oxidative reagents to enable its transformation into **3**. Disaccharide **4** could be prepared by stereoselective glycosylation of **8** with **9** and then regioselective deprotection.

Our synthesis started with the preparation of **5**–**9** according to reported procedures.[21] Thereafter, **7** was glycosylated with **6** using methyltriflate (MeOTf) as the promoter to provide disaccharide **10** (Scheme 2). The newly formed glycosidic

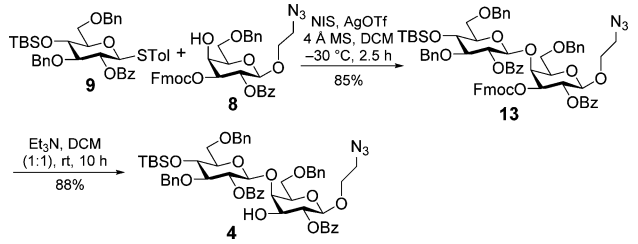
Scheme 2. Synthesis of the Trisaccharide Donor 3



linkage was proved to be β, judging from its H-1/H-2 coupling constant ($^3J_{H1,2}$ = 7.9 Hz). Then the fluorenylmethyloxycarbonyl (Fmoc) group in **10** was removed with triethylamine, followed by glycosylation of **11** with **5** using trimethylsilyl triflate (TMSOTf) as the promoter. The α-linkage of the sialyl residue in the resulting **12** was verified by its H-3a/C-1 coupling constant (6.5 Hz) derived from the single frequency off-resonance decoupling (SFORD) NMR spectrum, and the reaction gave only the α-anomer because of the presence of a trifluoroacetyl group at the 5-*N*-position in **5**.[20] Treatment of **12** with ceric ammonium nitrate (CAN) to remove the MP group and reaction of the resulting hemiacetal with trichloroacetonitrile in the presence of 1,8-diazabicycloundec-7-ene (DBU) afforded **3** as an anomeric mixture.

Compound **4** (Scheme 3) was obtained by glycosylation of **8** with thioglycoside **9** in the presence of *N*-iodosuccinimide (NIS)

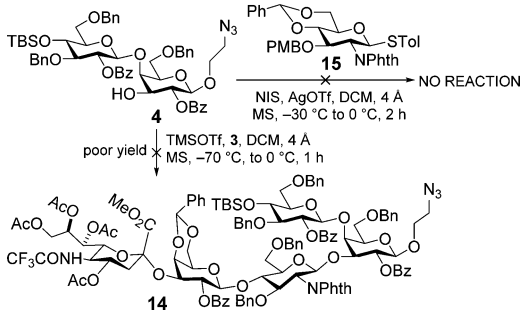
Scheme 3. Synthesis of the Disaccharide Acceptor 4



and silver triflate (AgOTf). Again, the reaction was stereospecific to form the desired β-glycosidic linkage. Subsequently, **13** was treated with triethylamine to remove the Fmoc protecting group and furnish compound **4**.

The glycosyl donor **3** and acceptor **4** were then subjected to [3 + 2] glycosylation (Scheme 4). However, the reaction gave little

Scheme 4. Glycosylation Reactions of 4

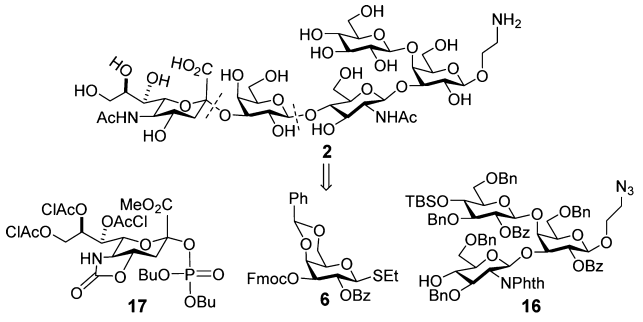


desired pentasaccharide **14**, which was observed by MS only. The majority of unreacted **4** and an elimination product of **3** were isolated. After several unsuccessful attempts to improve the reaction under various conditions, we began to suspect the reactivity of **4**. One question was whether, as commonly observed in the literature,[22,23] the galactopyranose 3-hydroxyl group is always reactive even in the presence of another sugar chain at its 4-*O*-position. Therefore, we probed the reaction between **4** and a simpler glucosyl donor **15**, which afforded no desired product either, indicating that **4** was probably very unreactive as a glycosyl acceptor.

Despite this finding, we had obtained useful information about the reactivity of glycosyl donor **4** involved in this synthesis. The above result clearly indicated that the presence of a sugar unit at the galactose 3-*O*-position would likely have a big impact on 4-*O*-glycosylation. This compelled us to revise our synthetic design and study a [3 + 1 + 1] strategy as shown in Scheme 5, in which we tried to make use of the synthesized intermediates as much as possible. Specifically, we wanted to first construct a branched trisaccharide **16** and then elongate the sugar chain via stepwise glycosylation with **6** and **17** as donors. Here, instead of **5**, sialoside **17**[24] was selected for the sialylation reaction due to the

DOI: 10.1021/ol5036563
Org. Lett. XXXX, XXX, XXX−XXX

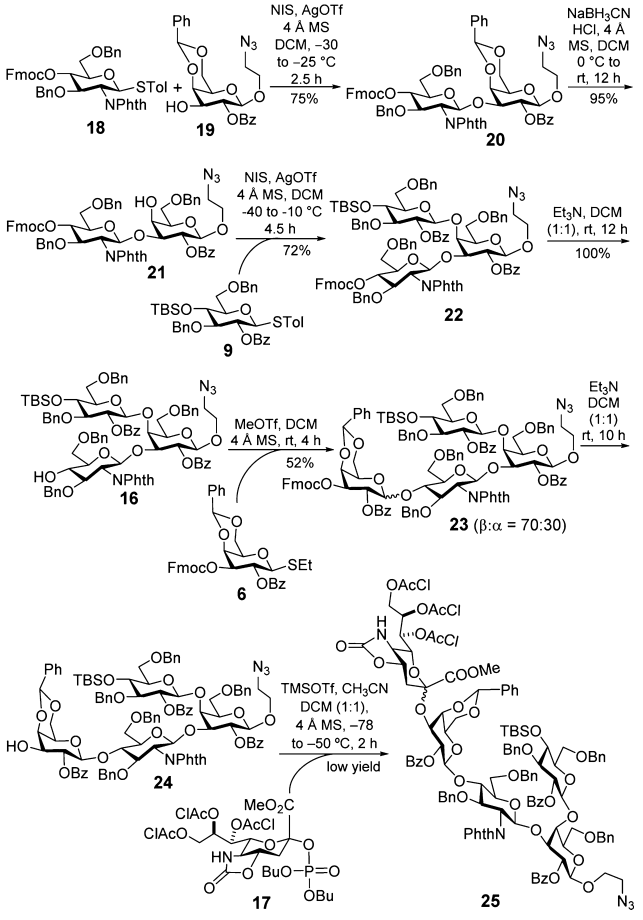
Scheme 5. Retrosynthesis of 2 by [3 + 1 + 1] Glycosylation



flexibility and efficiency that **17** had shown in another project in our laboratory.

The [3 + 1 + 1] synthesis commenced with the preparation of **16** (Scheme 6). Glycosylation of **18** with **19** in the presence of

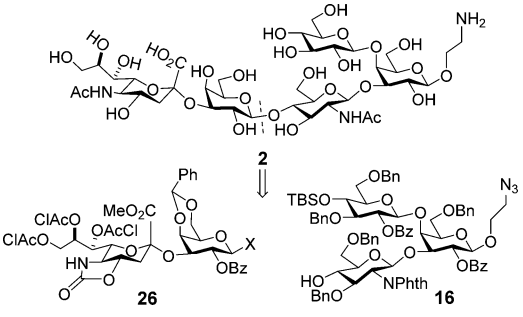
Scheme 6. Attempted Synthesis of 2 by the [3 + 1 + 1] Strategy



NIS/AgOTf afforded **20**. Thereafter, its benzylidene ring was regioselectively opened with $NaBH_3CN$ and HCl. Subsequent glycosylation of **21** with **9**, followed by the Fmoc group removal with triethylamine, gave **16**. The ^1H NMR spectrum of **16** showed that all its glycosidic linkages were β (δ 5.36, 5.26, and 5.23: $^3J_{H1,2}$ = 7.8, 7.8, and 7.8 Hz, respectively, in $CDCl_3$). This result indicated that the 4-OH group in **21** was quite reactive. The reaction between **16** and **6** produced a mixture of **23** (β:α = 70:30) despite the presence of a participating Bz protecting group at the 2-*O*-position of **6**, and the two isomers were difficult to separate. The mixture was thus directly subjected to Fmoc removal. Fortunately, the deprotected products were separable to get **24** that was fully characterized. Glycosylation of **24** with **17**[24] was performed with TMSOTf. According to MALDI MS analysis of the reaction mixture, only a small amount of the desired pentasaccharide **25** was formed, and the majority of **24** was retrieved together with its desilylated form. However, **25** and desilylated **24** were inseparable. In view of the low yield for the final key sialylation step and the difficulty with intermediate purification, we decided to switch to an alternative and possibly more effective synthetic plan.

On the basis of the success in preparing trisaccharide **16** and the glycosylation of **16** with **6** (Scheme 6), we envisioned a synthetic strategy that used [2 + 3] glycosylation (Scheme 7). In

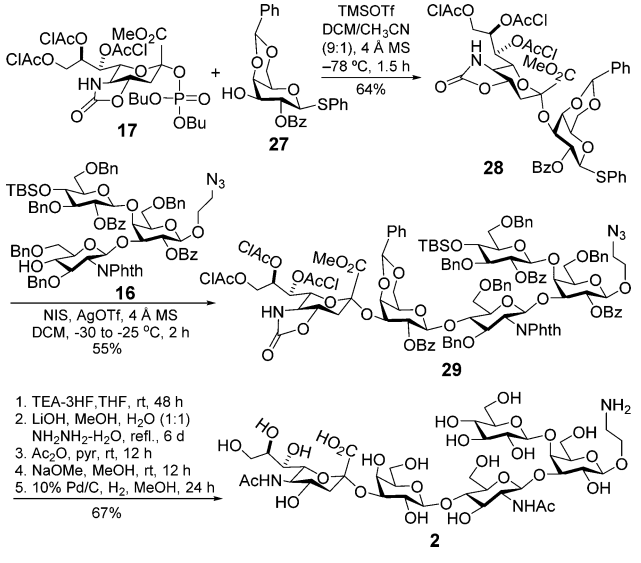
Scheme 7. Retrosynthesis of 2 by [2 + 3] Glycosylation



addition to trisaccharide fragment **17**, the other key building block was a disaccharide donor. This design was convergent and had the advantage of realizing the difficult sialylation at an early stage to avoid the problems depicted in Scheme 6.

Disaccharide donor **28** was prepared by stereospecific glycosylation of **27** with **17** in the presence of TMSOTf (Scheme 8). The stereochemistry of the newly formed glycosidic

Scheme 8. Successful Synthesis of the Target Molecule 2



bond was confirmed by SFORD NMR, showing that the coupling constant between sialic acid H-3a and its methyl ester carbonyl carbon was 6.5 Hz. Subsequently, the reaction between **28** and **16** under the influence of NIS and AgOTf furnished [2 + 3] glycosylation smoothly to afford the desired pentasaccharide **29** in a satisfactory yield (55%). Spectral analysis of **29** confirmed

DOI: 10.1021/ol5036563
Org. Lett. XXXX, XXX, XXX−XXX

the correct stereochemistry of the new glycosidic linkage (δ 4.83, $^3J_{H1,2}$ = 8.4 Hz in $CDCl_3$). It was interesting to observe that the reaction between **28** and **16** was more stereoselective than that between **6** and **16**. An untested explanation was that the presence of a bulky sialic acid unit at the 3-*O*-position in **28** might affect the conformation of the galactose residue and thus the stereochemical outcome of glycosylation.

With **29** in hand, the only remaining task was global deprotection and *N*-acetylation of the sugar rings, which turned out to be challenging. The protecting groups in **29** included a methyl carboxylate, a carbamate, a phthalyl group, several chloroacetyl (ClAc) and Bz groups, a TBS group, an azide, a benzylidene group, and multiple Bn groups. To successfully remove all of them, it was critical to employ the proper deprotection conditions and in the right sequence. After examining various combinations of reactions and conditions, we established the following effective protocol. First, **29** was stirred with Et_3N−HF in THF for 2 days to remove the TBS group. The resultant intermediate was then treated with LiOH in water and methanol (1:1) at 60 °C for 2 h to hydrolyze the methyl ester and to remove the ClAc, Bz, and carbamate groups, followed by heating at reflux with hydrazine for 6 d to remove the Phth group. The exposed amino groups and hydroxyl groups were acetylated with acetic anhydride and pyridine (1:1), and then the *O*-acetyl groups were selectively removed with sodium methoxide in methanol. Finally, Pd/C-catalyzed hydrogenolysis removed the benzyl and benzylidene groups and also reduced the azido group to afford the target molecule **2** (67% overall, five steps), which was purified on a Sephadex G-25 gel filtration column with distilled water as eluent and fully characterized with 1D, 2D NMR, and HR MS. All reactions were carried out consecutively without purification of the intermediates. However, all of the steps were closely monitored by TLC and MS. It is important that each reaction is complete, or the system becomes too complex for the final product purification.

In conclusion, the first chemical synthesis of the repeating unit of type Ia GBS CPS is described. Key to this successful synthesis was the proper installation of two sugar chains on the galactose 3- and 4-*O*-positions to create the branched structure. Investigation of the glycosylation reactions of galactose derivatives with free 3- or 4-hydroxyl groups revealed that the presence of a sugar at the 4-*O*-position had a big impact on the reactivity of the 3-hydroxyl group, that is, making the latter unreactive or inaccessible for further glycosylation. Glycosylating the 3-*O*-position had a smaller impact on the 4-*O*-position. As a result, the proper sequence for branch installation around galactose 3-/4-*O*-positions was to glycosylate the 3-*O*-position prior to 4-*O*-glycosylation. This may be generally suitable for the synthesis of other oligosaccharides with branched galactose, including many GBS CPSs. Moreover, introducing the sialic acid unit at the final synthetic stage poses a risk, which should be avoided. Ultimately, the synthesis was accomplished by a convergent [2 + 3] glycosylation strategy with sialodisaccharide **28** as the donor and branched trisaccharide **16** as the acceptor to address the above-mentioned problems. It is also worth mentioning that the designed target **2** had a free amino group at the oligosaccharide reducing end and that fully protected pentasaccharide **29** as the key synthetic intermediate had a distinguishable TBS protecting group at the glucose 4-*O*-position. This should facilitate the attachment of **2** to other molecules through selective reactions of its amino group to obtain synthetic vaccines, as well as elongation of the carbohydrate chain in **29** to obtain more complex structures.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures for compounds **3**−**25** and **27**−**29**; analytical and spectral characterization data of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +1-313-577-2557. E-mail: zwguo@chem.wayne.edu.

Author Contributions

†These authors contributed equally to the present work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by an NIH/NCI research grant (R01 CA95142). The 600 MHz NMR instrument was supported by an NSF grant (CHE-0840413). We thank Dr. Bashar Ksebati, Department of Chemistry, Wayne State University, for some of the NMR measurements.

■ REFERENCES

(1) Cieslewicz, M. J.; Chaffin, D.; Glusman, G.; Kasper, D.; Madan, A.; Rodrigues, S.; Fahey, J.; Wessels, M. R.; Rubens, C. E. *Infect. Immun.* **2005**, *73*, 3096.

(2) Howard, C. J.; Glynn, A. A. *Immunology* **1971**, *20*, 767.

(3) Hayrinen, J.; Bitter-Suermann, D.; Finne, J. *Mol. Immunol.* **1989**, *26*, 523.

(4) Wyle, F. A.; Artenstein, M. S.; Brandt, B. L.; Tramont, E. C.; Kasper, D. L.; Altieri, P. L.; Berman, S. L.; Lowenthal, J. P. *J. Infect. Dis.* **1972**, *126*, 514.

(5) Edwards, M. S.; Kasper, D. L.; Jennings, H. J.; Baker, C. J.; Nicholson-Weller, A. *J. Immunol.* **1982**, *128*, 1278.

(6) Kim, K. S.; Kang, J. H.; Cross, A. S. *FEMS Microbiol. Lett.* **1986**, *35*, 275.

(7) Cieslewicz, M. J.; Kasper, D. L.; Wang, Y.; Wessels, M. R. *J. Biol. Chem.* **2001**, *276*, 139.

(8) Guo, Z.; Boons, G.-J. *Carbohydrate-Based Vaccines and Immunotherapies*; Wiley: Hoboken, 2009.

(9) Anish, C.; Schumann, B.; Pereira, C. L.; Seeberger, P. H. *Chemi. Biol.* **2014**, *21*, 38.

(10) Goebel, W. F. *J. Exp. Med.* **1939**, *69*, 353.

(11) Goebel, W. F. *J. Exp. Med.* **1940**, *72*, 33.

(12) Ada, G.; Isaacs, D. *Clin. Microbiol. Infect.* **2003**, *9*, 79.

(13) Wessels, M. R. J. *Appl. Microbiol. Symp.* **1997**, *83* (Suppl), 20s.

(14) Astronomo, R. D.; Burton, D. R. *Nat. Rev. Drug Disc.* **2010**, *9*, 308.

(15) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

(16) Schmidt, R. R. *Angew. Chem., Int. Ed.* **1986**, *25*, 212.

(17) Roussel, F.; Knerr, L.; Grathwohl, M.; Schmidt, R. R. *Org. Lett.* **2000**, *2*, 3043.

(18) Pan, Y.; Chefalo, P.; Nagy, N.; Harding, C.; Guo, Z. *J. Med. Chem.* **2005**, *48*, 875.

(19) Yang, Y.; Li, Y.; Yu, B. *J. Am. Chem. Soc.* **2009**, *131*, 12076.

(20) Meo, C. D.; Demchenko, A. V.; Boons, G. J. *J. Org. Chem.* **2001**, *66*, 5490.

(21) Lindberg, J.; Svensson, S. C. T.; Påhlsson, P.; Konradsson, P. *Tetrahedron* **2002**, *58*, 5109.

(22) Moitessier, N.; Chapleur, Y. *Tetrahedron Lett.* **2003**, *44*, 1731.

(23) Haines, A. H. *Adv. Carbohydr. Chem. Biochem.* **1976**, *33*, 11.

(24) Chu, K. C.; Ren, C. T.; Lu, C. P.; Hsu, C. H.; Sun, T. H.; Han, J. L.; Pal, B.; Chao, T. A.; Lin, Y. F.; Wu, S. H.; Wong, C. H.; Wu, C. Y. *Angew. Chem., Int. Ed.* **2011**, *50*, 9391.

DOI: 10.1021/ol5036563
Org. Lett. XXXX, XXX, XXX−XXX