Organic LETTERS

Letter

pubs.acs.org/OrgLett

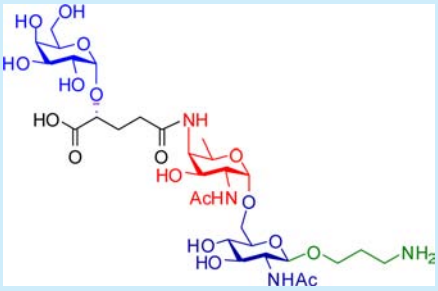
First Synthesis of *Bacillus cereus* Ch HF-PS Cell Wall Trisaccharide Repeating Unit

Ananda Rao Podilapu and Suvarn S. Kulkarni*

Department of Chemistry, Indian Institute of Technology Bombay, Mumbai 400076, India

Supporting Information

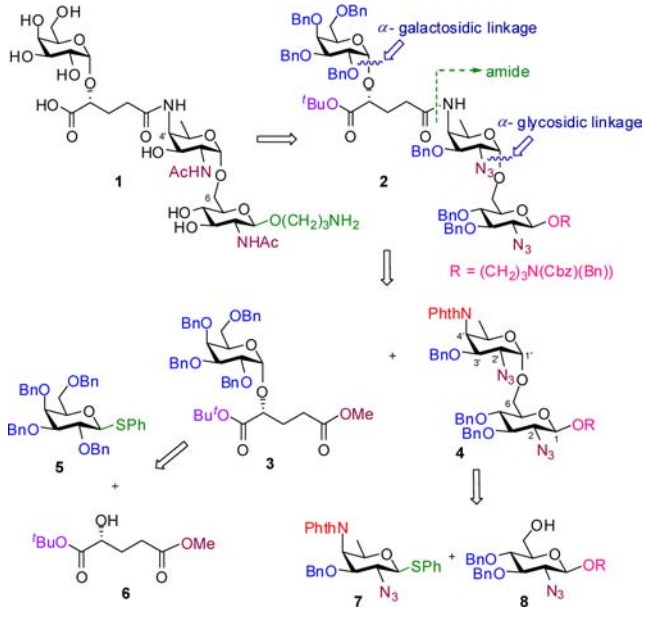
ABSTRACT: The first total synthesis of Ch HF-PS, a cell wall trisaccharide repeating unit of *B. cereus*, is reported. The synthetic trisaccharide is appended with an aminopropyl linker at the reducing end to allow for conjugation to proteins and microarrays. The convergent synthesis involves transformation of D-mannose into an orthogonally protected rare AAT sugar building block, two consecutive α-stereoselective glycosylations, β-selective attachment of the linker by solvent participation, and amide bond formation, as key steps.



Bacillus cereus is an aerobic, Gram-positive, spore-forming bacterium which is a causative agent of self-limiting food poisoning syndrome.[1] The symptoms of the acute gastrointestinal illness include nausea, abdominal cramps, diarrhea, and occasional vomiting and fever. The ubiquitous bacterium which was once thought to be benign is increasingly being acknowledged as a human pathogen. *B. cereus* can cause serious opportunistic infections such as wound infections, bacteremia, septicaemia, meningitis, pneumonia, infections of the central nervous system, endocarditis, pericarditis, respiratory infections, and peripheral infections.[1−4] It is also associated with keratitis, panopthalmitis, and other ocular ophthalmic infections which usually result in the loss of the eye.[5] *B. cereus* infections in immunocompromised individuals may be lethal.[6] Moreover, inhalative exposure to *B. cereus*, containing *B. anthracis* toxin genes, causes severe fatal pneumonia in healthy individuals.[7] Furthermore, *B. cereus* infections are difficult to treat, as the bacterium is resistant to several antibiotics including penicillin, ampicillin, cephalosporins, and trimethoprim.[8] Currently there are no vaccines against *B. cereus* mediated infectious diseases.

Gram-positive bacteria synthesize various secondary cell wall polysaccharides (SCWPs) which are covalently attached to the peptidoglycan of the bacterial cell wall.[9] These SCWPs mediate various important functions such as bacterial adhesion, host immunomodulation, virulence, and biofilm formation and are thus regarded as potential carbohydrate antigens for the development of vaccines as well as serodiagnostic markers. Recently, Guérardel and co-workers analyzed *B. cereus* ATCC 14579 and characterized two structurally unrelated SCWPs named as Ne HF-PS and Ch HF-PS.[10] Of these, the charged glycan Ch HF-PS (Scheme 1) is made up of a completely original sequence, →6)-Gal(α1−2)(2-*R*-hydroxyglutar-5-ylamido)Fuc2NAc4N(α1−6)GlcNAc(β1→, identified in the *Bacillus* genus for the first time. A unique structural feature of Ch HF-PS is the presence of the rare sugar and the hydroxyglutaric acid spacer which imparts charge and flexibility

Scheme 1. Retrosynthetic Analysis



to the glycan. Due to its unprecedented structure and immunogenic potential, the trisaccharide repeating unit is an attractive synthetic target. Over the past few years there have been remarkable advances in the identification[11,12] and synthesis of rare sugar-containing bacterial glycans.[13] The total synthesis of a *B. cereus* glycan has yet to be reported. In continuation of our research program on the synthesis of the rare sugar containing bacterial glycoconjugates,[14] we report herein a first total synthesis of the unusual trisaccharide motif **1**

Received: July 22, 2014
Published: August 7, 2014

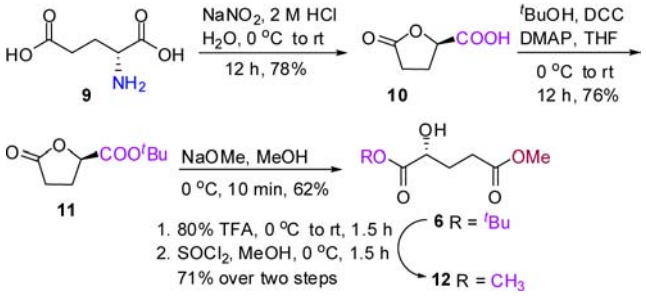
dx.doi.org/10.1021/ol5021527 | *Org. Lett.* 2014, 16, 4336−4339

(Scheme 1). The synthetic trisaccharide is appended with a terminal aminopropyl linker for subsequent conjugation to a carrier protein or immobilization onto a microarray.

Structurally, target molecule **1** is composed of a terminal D-galactopyranose α-linked to a 2-(*R*)-hydroxy-D-glutaric acid moiety, which in turn is attached via an amide bond to the rare sugar 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT/Fuc2NAc4N) at C4′, which is further connected to the linker-attached D-glucosamine unit at O6. The key challenges encountered in the synthesis of trisaccharide **1** are the synthesis of the orthogonally protected rare sugar AAT building block, β-selective attachment of the linker, and installation of two consecutive α-glycosidic linkages. Retrosynthetically, the most obvious disconnection is the amide bond in the fully protected trisaccharide **2**. This dissects the molecule into two halves **3** and **4**, which can in principle be separately synthesized and coupled together, in a convergent manner, by selectively exposing the acid and amine functionalities, respectively, to arrive at **2**. The left-hand unit **3** could be synthesized by stereoselective glycosylation of easily accessible D-galactosyl donor **5** with orthogonally protected 2-(*R*)-hydroxyl D-glutaric acid acceptor **6**, under appropriate conditions. The key disaccharide **4** could potentially be assembled by α-selective coupling of the orthogonally protected AAT thioglycoside donor **7** with the linker attached 6-OH glucosamine acceptor **8**. The selection of the protecting group in disaccharide **4** would demand careful attention. Since the terminal C4′-NH_2 group needs to be revealed selectively postglycosylation, a phthalimido protection was deemed suitable. The remaining C2 and C2′ NH_2 groups present on D-glucosamine and AAT units, respectively, were masked as azido groups. While the C2′ azido group in **7** was expected to favor α-selectivity, the C2 azido group in **8** restricted the choice to solvent participation for obtaining β-selectivity with the linker acceptor. Finally, to maintain orthogonality, we opted for a (benzyl)benzyloxycarbonyl-amino linker that had been previously employed in oligosaccharide synthesis.[15] The hydroxyl groups in **7** and **8** were masked as benzyl ethers for enhancing the reactivity of the donors in glycosylation. Moreover, benzyl groups are stable under all the glycosylation and phthalimide deprotection conditions and can be conveniently removed by hydrogenolysis at the end.

Our synthesis started with the preparation of orthogonally protected 2-(*R*)-hydroxy D-glutaric acid unit **6** (Scheme 2). Readily available D-glutamic acid **9** was converted to (*R*)-butyrolactone **10** (78%) following the procedure reported by Austin and co-workers.[16] Esterification of **10** was carried out by DCC mediated coupling with *t*-BuOH to obtain **11** (76%), which was subjected to lactone ring opening by NaOMe in MeOH to afford **6** (62%) (Scheme 2). The absolute stereochemistry of alcohol **6** was confirmed by converting it to a known derivative **12** by selective hydrolysis of the *tert*-butyl ester under acidic conditions followed by esterification with methanol. The optical rotation of **12** matched perfectly well with the reported value,[17] confirming *R* stereochemistry in **6** (see Supporting Information).

Scheme 2. Preparation of D-Glutaric Acid Derivative



Synthesis of the left-hand unit **3** using known thioglycoside donor **5** and acceptor **6** was attempted next. Obtaining α-selectivity in galactosylation is a challenging task. For this reason, we screened various sets of reaction conditions, varying the leaving groups, additives, and reaction temperatures (Table 1). The solvent participation effect was explored first, using

Table 1. α-Stereoselective Glycosylation of 5 and 6

entry	conditions	T (°C)	time	α/β	yield (%)
1	NIS, TMSOTf CH_2Cl_2/Et_2O 1:1	−20	2 h	8:1	48
2	NIS, TMSOTf CH_2Cl_2/Et_2O 1:1	−40	2 h	9:1	50
3	NIS, TMSOTf CH_2Cl_2/Et_2O 1:1	−70	2 h	12:1	62
4	1. NBS, THF, H_2O 2. K_2CO_3, CCl_3CN 3. TMSOTf CH_2Cl_2/Et_2O 1:1	−70	1 h	–	–
5	1. Br_2, CH_2Cl_2 2. AgOTf, CH_2Cl_2	−50/−30	1 h	–	–
6	1. Br_2, CH_2Cl_2 2. TBAI	40	3 d	1:0	25
7	**Ph_2SO, Tf_2O, CH_2Cl_2**	**−60**	**1 h**	**1:0**	**75**

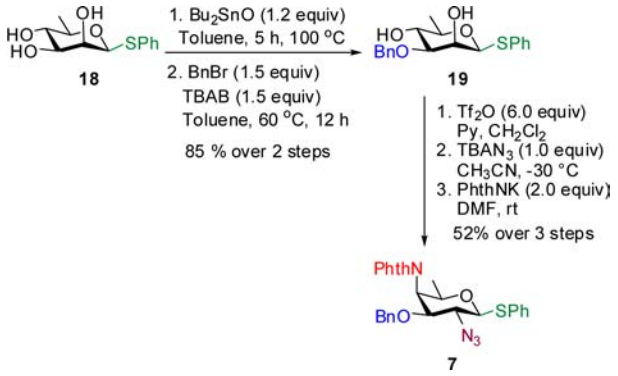
diethyl ether as a cosolvent (entries 1−3).[18] Glycosylation of thioglycoside **5** under NIS/TMSOTf activation conditions in a dichloromethane/diethyl ether solvent mixture (1:1) with acceptor **6** at −20 °C afforded the desired product **3** with good selectivity (8:1). Lowering of the temperature to −40 °C (entry 2) and −70 °C (entry 3), under identical conditions, progressively improved the α/β selectivity to 9:1 and 12:1, respectively. Since the two isomers could not be separated by silica gel flash column chromatography even after repeated attempts, it became imperative for us to tune the conditions so as to obtain a single α-isomer. Thioglycoside **5** being a stable and flexible donor could be readily converted into the corresponding trichloroacetimidate (−O-C(NH)CCl_3)[19] and bromide (entries 4 and 5). However, the trichloroacetimidate donor was too reactive and decomposed spontaneously under the reaction conditions even at −70 °C (entry 4). Similarly, AgOTf promoted the activation of the corresponding galactosyl bromide at −30 and −50 °C in the presence of acceptor **6** led to complex mixtures (entry 5). We did observe clean α-selectivity under *in situ* anomerization conditions[20] using TBAI to obtain pure **3**, albeit in a low yield and after prolonged stirring at 40 °C (entry 6). This outcome was still far from

dx.doi.org/10.1021/ol5021527 | Org. Lett. 2014, 16, 4336−4339

satisfactory from a practical point of view. Gratifyingly, diphenylsulfoxide and Tf_2O mediated[21] thioglycoside activation of **5** at −60 °C with acceptor **6** rapidly furnished only α-linked product **3** (^{1}H NMR H-1 δ 5.12, J = 3.9 Hz, ^{13}C NMR C-1 δ 95.8) in 75% yield (entry 7). Selective deprotection of the methyl ester in **3** under mild basic conditions furnished acid **13** (85%).

The synthesis of the key AAT building block **7** from known D-rhamnosyl triol **18**[14b] is outlined in Scheme 3. Earlier, we had

Scheme 3. Synthesis of the Orthogonally Protected AAT Building Block

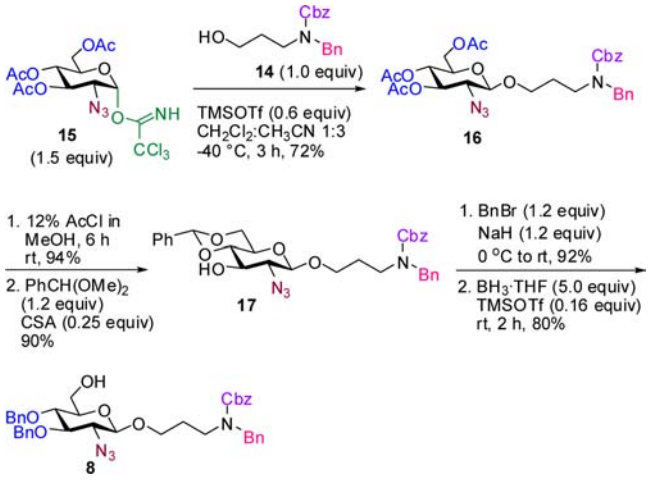


successfully synthesized a 3-OBz derivative corresponding to **7** from **18**.[14b] However, since the 3-OBz group was not compatible with phthalimide deprotection conditions, we decided to employ a 3-OBn derivative instead. Accordingly, triol **3** was first converted to the corresponding tin ketal by treatment with dibutyltin oxide in toluene at 100 °C and further reacted with benzyl bromide and TBAB at 60 °C to afford the 3-OBn derivative **19**, in high regioselectivity, as a single isomer in 85% yield over two steps. Following our recently established protocol of regioselective nucleophilic displacements of triflates,[14b] the 2,4-diol **19** was first converted to the corresponding 2,4-bis-triflate by treatment with Tf_2O in pyridine. The crude triflate obtained after aqueous workup was subjected to a regioselective C2-OTf displacement with a stoichiometric amount of $TBAN_3$ at −30 °C. After the complete conversion of the starting material as observed from TLC analysis, potassium phthalimide was added in the same pot at rt to displace the remaining C4-OTf to afford the desired orthogonally protected AAT building block **7** in 52% yield over three steps after a single chromatographic purification.

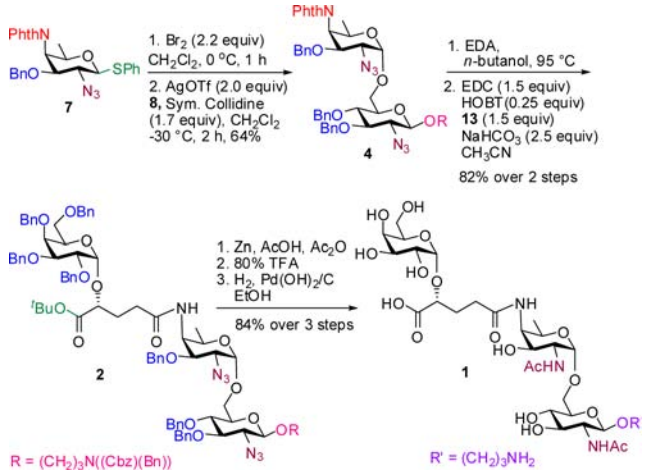
The linker attached reducing end D-glucosamine building block **8** was prepared from known trichloroacetimidate donor **15**[15c] and linker **14**[15d] as shown in Scheme 4. Glycosylation of **15** with **14** under TMSOTf activation using acetonitrile as a participating solvent[22] at −40 °C cleanly afforded the β-linked product **16** (^{1}H NMR H-1 δ4.33, ^{13}C NMR C-1 δ 102.06) in 72% yield. Removal of the acetates under mild conditions (AcCl, MeOH, 94%) followed by benzylidene formation in acetonitrile[23] as a solvent (90%) furnished 3-OH derivative **17** which was benzylated (92%) and subjected to a regioselective BH_3-reductive ring opening reaction[24] (80%) to fashion the requisite 6-OH acceptor **8**.

Assembly of the target molecule, its functional group transformation, and global deprotection are delineated in Scheme 5. The key AAT thioglycoside **7** was converted to the corresponding glycosyl bromide in situ and treated with

Scheme 4. Synthesis of the Linker Attached Reducing End 6-OH D-Glucosamine Unit



Scheme 5. Assembly of the Trisaccharide and Global Deprotection



acceptor **8** in the presence of AgOTf at −30 °C to cleanly afford the desired α-linked disaccharide **4** as a single isomer in 64% yield. Selective removal of the phthalimide protecting group using ethylene diamine in *n*-BuOH at an elevated temperature (95 °C)[25] and its subsequent EDC-HOBT[26] mediated coupling with acid **13** afforded the fully protected trisaccharide **2** (82% over two steps). Global deprotection of **2** via azide to NHAc conversion and hydrolysis of the *tert*-butyl ester, followed by hydrogenolytic removal of benzyl groups and purification on Sephadex G-25, furnished the target trisaccharide **1** (84% over three steps). Target molecule **1** and all the synthetic intermediates were thoroughly characterized by ^{1}H, ^{13}C, and 2D NMR as well as HRMS (see Supporting Information).

In conclusion, we have reported the first total synthesis of a Ch HF-PS trisaccharide repeating unit from *B. cereus*. The glycan is equipped with a β-O-linked aminopropyl linker at the reducing end to allow for further attachment to carrier proteins. Our convergent approach also enables rapid synthesis of the rare sugar containing disaccharide. Immunological studies of **1** and its derivatives may provide valuable insight regarding the feasibility of developing a carbohydrate-based vaccine and serodiagnostic markers for *B. cereus*.

dx.doi.org/10.1021/ol5021527 | *Org. Lett.* 2014, 16, 4336−4339

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and procedures, compound characterization data, and copies of ^{1}H and ^{13}C spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: suvarn@chem.iitb.ac.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Department of Science and Technology (Grant No. SR/S1/OC-40/2009), Council of Scientific and Industrial Research (Grant No. 01(2376)/10/EMR-II), and Board of Research in Nuclear Sciences (Grant No. 2013/37C/51/BRNS) for financial support. A.R.P. thanks CSIR-New Delhi for a fellowship.

■ REFERENCES

(1) Public health agency of Canada. http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/bacillus-cereus-eng.php.

(2) Logan, N. A.; Rodrigez-Diaz, M. Bacillus spp. and Related Genera. In *Principles and Practice of Clinical Bacteriology*, 2nd ed.; Gillespie, S. H., Hawkey, P. M., Eds.; John Wiley and Sons Ltd.: West Sussex, England, U.K., 2006; pp 139−158.

(3) Rosovitz, M. J.; Voskuil, M. I.; Chambliss, G. H. Bacillus. In *Topley & Wilson's Microbiology and Microbial Infection: Systematic Bacteriology*, 9th ed.; Collier, L., Balows, A., Sussman, M., Balows, A., Duerden, B. I., Eds.; Hodder Education Publishers (Arnold): USA, 1998; pp 709−729.

(4) Drobniewski, F. A. *Clin. Microbiol. Rev.* **1993**, *6*, 324−338.

(5) (a) Pinna, A.; Sechi, L. A.; Zanetti, S.; Delogu, G.; Cappuccinelli, P.; Carta, F. *Ophthalmology* **2001**, *108*, 1830−1834. (b) Cowan, C. L., Jr.; Madden, W. M.; Hatem, G. F.; Merritt, J. C. *Ophthalmology* **1987**, *19*, 65−68. (c) O'Day, D. M.; Smith, R. S.; Gregg, C. R.; Turnbull, P. C.; Head, W. S.; Ives, J. A.; Ho, P. C. *Ophthalmology* **1981**, *88*, 833−838.

(6) Le Scanff, J.; Mohammedi, I.; Thiebaut, A.; Martin, O.; Argaud, L.; Robert, D. *Infection* **2006**, *34*, 98−99.

(7) Avashia, S. B.; Riggins, W. S.; Lindley, C.; Hoffmaster, A.; Drumgoole, R.; Nekomoto, T.; Jackson, P. J.; Hill, K. K.; Williams, K.; Lehman, L.; Libal, M. C.; Wilkins, P. P.; Alexander, J.; Tvaryanas, A.; Betz, T. *Clin. Infect. Dis.* **2007**, *44*, 414−416.

(8) Ginsburg, A. S.; Salazar, L. G.; True, L. D.; Disis, M. L. *Am. J. Hematol.* **2003**, *72*, 204−208.

(9) Vollmer, W. *Microbiol. Rev.* **2008**, *32*, 287−306.

(10) Candela, T.; Maes, E.; Garénaux, E.; Rombouts, Y.; Krzewinski, F.; Gohar, M.; Guérardel, Y. *J. Biol. Chem.* **2011**, *286*, 31250−31262.

(11) Adibekian, A.; Stallforth, P.; Hecht, M.-L.; Werz, D. B.; Gagneux, P.; Seeberger, P. H. *Chem. Sci.* **2011**, *2*, 337−344.

(12) (a) Dube, D. H.; Champasa, K.; Wang, B. *Chem. Commun.* **2011**, *47*, 87−101. (b) Longwell, S. A.; Dube, D. H. *Curr. Opin. Chem. Biol.* **2013**, *17*, 41−48. (c) Tra, V. N.; Dube, D. H. *Chem. Commun.* **2014**, *50*, 4659−4673.

(13) Emmadi, M.; Kulkarni, S. S. *Nat. Prod. Rep.* **2014**, *31*, 870−879.

(14) (a) Emmadi, M.; Kulkarni, S. S. *Org. Biomol. Chem.* **2013**, *11*, 3098−3102. (b) Emmadi, M.; Kulkarni, S. S. *Nat. Protoc.* **2013**, *8*, 1870−1889. (c) Emmadi, M.; Kulkarni, S. S. *Carbohydr. Res.* **2014**, DOI: 10.1016/j.carres.2014.04.011. (d) Emmadi, M.; Kulkarni, S. S. *Org. Biomol. Chem.* **2013**, *11*, 4825−4830.

(15) (a) Mong, T. K.-K.; Lee, H.-K.; Durón, S. G.; Wong, C.-H. *Proc. Natl. Acad. Sci. Lett.* **2003**, *100*, 797−802. (b) Wang, Y. H.; Huang, X. F.; Zhang, L. H.; Ye, X. S. *Org. Lett.* **2004**, *6*, 4415−4417. (c) Arungundram, S.; Al-Mafraji, K.; Asong, J.; Leach, F. E.; Amster, J.; Venot, A.; Turnbull, J. E.; Boons, G. J. *J. Am. Chem. Soc.* **2009**, *131*, 17394−17405. (d) Noti, C.; de Paz, J. L.; Polito, L.; Seeberger, P. H. *Chem.—Eur. J.* **2006**, *12*, 8664−8686.

(16) Austin, A. T.; Howard, J. *J. Am. Chem. Soc.* **1954**, *76*, 3593−3603.

(17) Whitman, C. P.; Hajipour, G.; Watson, R. J.; Johnson, W. H.; Bembenek, M. E.; Stolowicht, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 10104−10110.

(18) Demchenko, A. V. General aspects of the glycoside bond formation. *In Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Demchenko, A. V., Ed.; Wiley-VCH: 2008; pp 1−27.

(19) Schmidt, R. R.; Kinzy, W. In *Advances in Carbohydrate Chemistry and Biochemistry*; Horton, D., Ed.; Academic Press: San Diego, 1994; Vol. *50*, p 21.

(20) Kulkarni, S. S.; Gervay-Hague, J. Glycosyl Chlorides, Bromides and Iodides. *In Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*; Demchenko, A. V., Ed.; Wiley-VCH: 2008; pp 59−93.

(21) Cato, D.; Buskas, T.; Boons, G. J. *J. Carbohydr. Chem.* **2009**, *74*, 6064−6071.

(22) Bracinni, I.; Derouet, C.; Esnault, J.; Herv'e de Penhoat, C.; Mallet, J. M.; Michon, V.; Sinaÿ, P. *Carbohydr. Res.* **1993**, *246*, 23−41.

(23) (a) Emmadi, M.; Kulkarni, S. S. *J. Org. Chem.* **2011**, *76*, 4703−4709. (b) Sanapala, S. S.; Kulkarni, S. S. *Chem.—Eur. J.* **2014**, *20*, 3578−3583.

(24) Wang, C.-C.; Lee, J.-C.; Luo, S.-Y.; Kulkarni, S. S.; Huang, Y.-W.; Lee, C.-C.; Chang, K.-L.; Hung, S.-C. *Nature* **2007**, *446*, 896−899.

(25) Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139−164.

(26) Ren, C.-T.; Chen, C.-S.; Wu, S.-H. *J. Org. Chem.* **2002**, *67*, 1376−1379.