Organic LETTERS

Letter

Cite This: Org. Lett. XXXX, XXX, XXX−XXX

pubs.acs.org/OrgLett

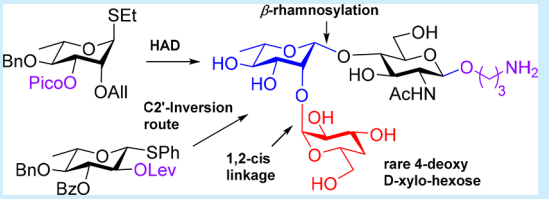
Total Synthesis of Trisaccharide Repeating Unit of *O*-Specific Polysaccharide of *Pseudomonas fluorescens* BIM B-582

Archanamayee Behera, Diksha Rai, Divya Kushwaha, and Suvarn S. Kulkarni*

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

Supporting Information

ABSTRACT: The first total synthesis of the trisaccharide repeating unit of the *O*-specific polysaccharide of *Pseudomonas fluorescens* BIM B-582 is reported. This efficient synthesis involves consecutive 1,2-*cis* glycosylations including β-L-rhamnosylation and α selective coupling of rare 4-deoxy-D-*xylo*-hexose as the key steps. The synthetic trisaccharide is equipped with an aminopropyl linker at the reducing end to allow for conjugation to proteins and microarrays for further immunological studies.



Pseudomonas fluorescens, a typical representative of the widespread genus *Pseudomonas* from the family Pseudomonadaceae, is a Gram-negative rod-shaped bacterium found in soil and water. *P. fluorescens* possesses hemolytic activity which may affect blood transfusion, especially in immunocompromised patients.[1] This pathogen is also one of the important phytopathogens which causes severe bacteriosis in a wide range of cultured plants and also causes spoilage of food, including milk, cheese, and meat.[2,3] *P. fluorescens* is known to form biofilms,[4] which are resistant to a variety of antibiotics. Thus, alternative methods to eradicate bacteria in the biofilm form, such as phage treatment, where the *O*-antigen is the major target, have been suggested.[5,6] Availability of the *O*-antigen would enable studies toward delineating the mechanism of the interaction between the phages and bacteria.

In 2011, Knirel and co-workers[7] isolated a major *O*-specific polysaccharide from the lipopolysaccharide of *P. fluorescens* BIM B-582. A unique feature of this polysaccharide is that it contains a novel and hitherto unknown rare sugar component, 4-deoxy-D-xylo-hexose (D-4dxylHex), as a lateral substituent in 40% of the oligosaccharide repeating units. The polysaccharide contains a disaccharide →3)-β-L-Rha*p*-(1 → 4)-β-D-Glc*p*NAc-(1→ backbone repeat with some of the β-L-rhamnose units appended at O2 with the α-D-4dxylHex*p*. The rare sugar-containing trisaccharide repeating unit (Figure 1) is crucial for bacterial specificity and therefore is an important synthetic target.

Structurally, the trisaccharide is composed of a terminal 4-deoxy-D-*xylo*-hexose (4-deoxy-D-glucose) unit which is α linked to L-rhamnose at O2, which in turn, is further β linked to the D-glucosamine moiety at O4. The construction of this trisaccharide repeating unit would encounter two major challenges. First, installation of the β-L-rhamnopyranosidic linkage[8] would present a well-known difficulty due to an unfavorable anomeric effect and steric hindrance from the C2 axial group which also would preclude neighboring group participation. Moreover, a conformational restriction approach was ruled out because of its 6-deoxy structure, which prevents use of a 4,6-*O*-benzylidene acetal[9] to restrict the conformation, to selectively install the desired β glycosidic bond. Another important challenge would be synthesis of the 4-deoxy-D-xyloHex*p* building block and its α selective coupling with the 2-OH of L-rhamnose. The consecutive 1−2 *cis* linkages on the adjacent 1,2-positions of L-rhamnose make the synthesis of this trisaccharide repeating unit challenging.

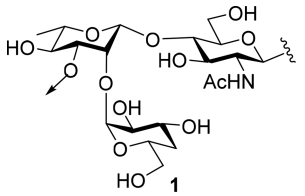


Figure 1. Structure of *O*-specific polysaccharide repeating unit.

Our retrosynthetic approach for the repeating trisaccharide unit of *P. fluorescens* is outlined in Figure 2. The target molecule **1** could potentially be obtained by debenzoylation and hydrogenolysis of fully protected linker appended trisaccharide **2**, which could be assembled by α selective coupling of 4-deoxy thioglycoside donor **4** and disaccharide acceptor **3**. The 4-deoxy donor **4** could, in turn, be obtained from commercially available D-galactose. Disaccharide **3** might be synthesized by glycosylation of a suitably protected L-quinovose donor **5** with glucosamine acceptor **6** followed by a C2′ inversion. Compound **5** could be obtained by C2 epimerization of a semiprotected L-rhamnose derivative. Alternatively, a direct stereoselective β-rhamnosylation with a suitably protected L-rhamnosyl donor with acceptor **6** would afford the disaccharide **3**. The D-glucosamine acceptor **6** might possibly be assembled from thioglycoside donor **7** and linker derivative **8**. Glucosamine derivative **7** would be obtained via a C2 inversion of commercially available D-mannose,[10] while the linker derivative

Received: August 21, 2018

DOI: 10.1021/acs.orglett.8b02669
Org. Lett. XXXX, XXX, XXX−XXX

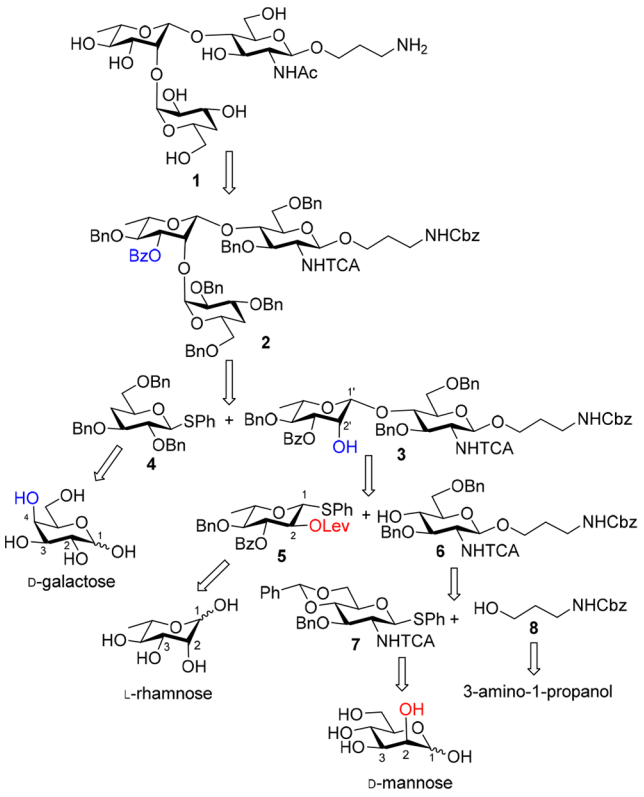
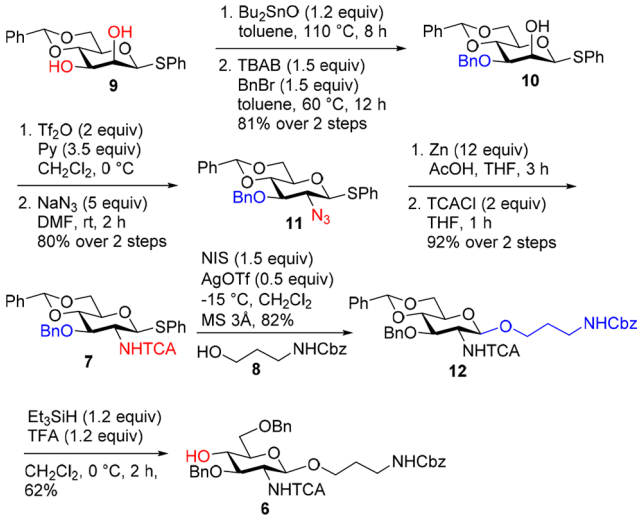


Figure 2. Retrosynthetic analysis.

8 could be prepared from commercially available 3-amino-1-propanol. With these considerations in mind, we began the total synthesis of **1**.

Our synthesis of the reducing end 4-OH glucosamine acceptor **6** from commercially available and abundant D-mannose is shown in Scheme 1. The easily accessible 2,3-diol

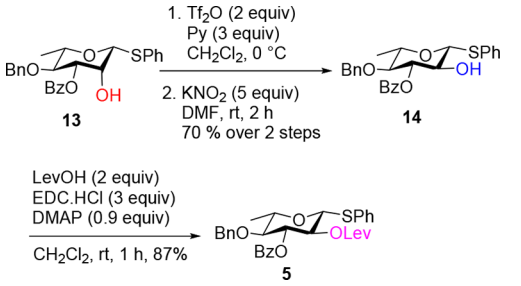
Scheme 1. Synthesis of D-Glucosamine Acceptor 6



9[10] was first converted to the corresponding tin ketal by treatment with dibutyltin oxide in toluene at 110 °C and was further reacted with benzyl bromide and tetrabutylammonium bromide (TBAB) at 60 °C to afford the 3-OBn derivative **10**, in high regioselectivity, as a single isomer in 81% yield over two steps. The C2-OH was subjected to triflation using triflic anhydride in pyridine, and the so-formed *O*-triflate was displaced by NaN_3 to furnish glucosamine derivative **11** in 80% yield over two steps. Reduction of the azide in **11** to the amine using Zn and AcOH followed by trichloroacetyl protection furnished thioglycoside donor **7** in 92% yield over two steps.

Glycosylation of donor **7**, with linker derivative **8** using NIS and AgOTf as a promoter at −15 °C gave *β*-linked glycoside **12** in 82% yield as a sole product. Reductive benzylidene ring opening of **12** using TFA and Et_3SiH furnished 4-OH glucosamine acceptor **6** in 62% yield. We first explored the synthesis of the key disaccharide backbone repeating unit employing indirect installation of the 1,2-*cis* rhamnosyl linkage via a double S_N2 inversion. For this purpose, the orthogonally protected rare L-quinovose derivative **5** was synthesized from L-rhamnose along the lines of our previously established protocol (Scheme 2).[11]

Scheme 2. Synthesis of L-Quinovose Donor 5

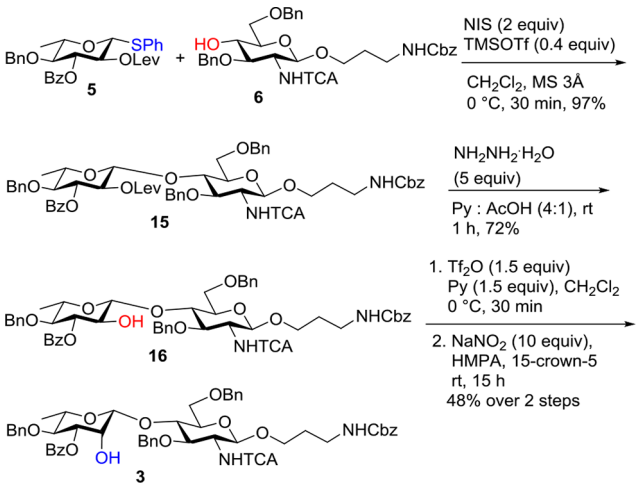


L-Quinovose is a C2 epimer of L-rhamnose. Alcohol **13** was obtained by selective 3-*O*-benzoylation of the easily accessible L-rhamnosyl 2,3-diol under tin-mediated conditions.[12] Triflation of the 2-OH of **13**, followed by S_N2 displacement of the formed C2-OTf by the nitrite anion, smoothly furnished L-quinovoside derivative **14** in 70% yield over two steps. It should be noted that the reaction worked well with potassium nitrite even without addition of a crown ether. The ease of S_N2 displacement of OTf in this case is in accordance with the proposed Hale−Hough−Richardson rules for nucleophilic displacement of pyranosidic *O*-triflates.[13] The free 2-OH in **14** was capped with a levulinoyl group upon treatment with levulinic acid, EDC·HCl, and DMAP, which afforded fully protected L-quinovoside donor **5** in 87% yield.

With all of the desired building blocks in hand, we went ahead with the coupling of **5** and **6** as outlined in Scheme 3. L-Quinovoside donor **5** and D-glucosamine acceptor **6** were glycosylated in the presence of NIS and TMSOTf as a promoter at 0 °C to deliver the *β*-linked disaccharide **15** in 97% yield as a single isomer. The selectivity can be attributed to the neighboring group participation of the C2 ester group in **5**. Regioselective deprotection of the levulinoyl group using $NH_2NH_2{\cdot}H_2O$ afforded disaccharide **16** in 72% yield. The equatorial 2′-OH in **16** was then epimerized back to axial 2′-OH to obtain the 1,2-*cis*-L-rhamno configuration. Accordingly, the free 2′-OH was subjected to *O*-triflation using triflic anhydride and pyridine, and subsequently, the formed *O*-triflate was treated with $NaNO_2$, HMPA, and 15-crown-5 to furnish the desired disaccharide acceptor **3** in 48% yield over two steps with clean selectivity. Along with the product, surprisingly, we also observed glycosidic bond cleavage and around 20% glucosamine acceptor **6** (confirmed from ^1H NMR, HRMS, and TLC

DOI: 10.1021/acs.orglett.8b02669
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis of Disaccharide Acceptor 3

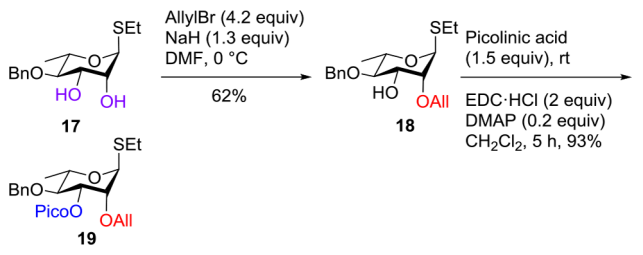


standard) was recovered, which could be recycled back for the coupling reactions.

Alternatively, we attempted the more challenging direct β-rhamnosylation route. Recently, Demchenko and co-workers established a picolinoyl group mediated hydrogen bond aglycon delivery (HAD) method for creating 1,2-*cis* glycosidic linkages with high selectivity.[8d] Using this method, they report a 1:25 α/β selectivity in the glycosylation of an L-rhamnose sugar with a highly reactive 6-OH glucosyl acceptor when the Pico group is placed at the O3 position. Although in our case we needed to glycosylate a poorly reactive 4-OH glucosamine acceptor, we decided to try out their conditions.

Thus, we went ahead with the synthesis of the 3-*O*-picoloyl donor **19** as delineated in Scheme 4. Diol **17** was synthesized on gram scale from commercially available and abundant starting material L-rhamnose over six steps in good yield.[14]

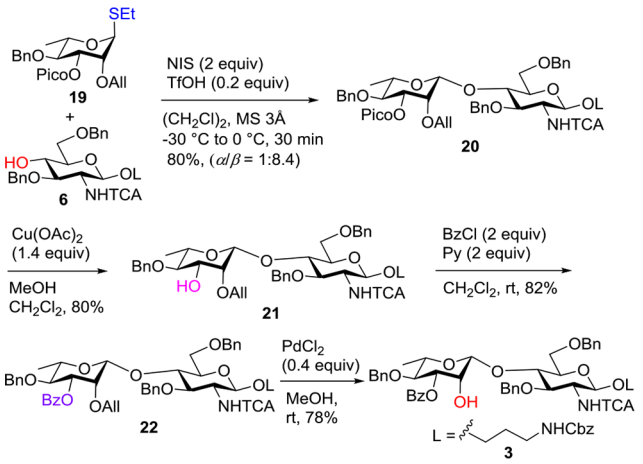
Scheme 4. Synthesis of Pico Donor 19



Regioselective allylation of diol **17** using AllylBr and NaH at 0 °C afforded 3-OH derivative **18** in 62% yield (along with 15% diallylated product). The free OH in **18** was capped with the picoloyl group via an EDC·HCl-mediated coupling with picolinic acid to obtain pico donor **19** in 93% yield.

Having both the donor **19** and acceptor **6** in hand, we proceeded with the picoloyl (HAD)-mediated glycosylation for the synthesis of our key disaccharide as described in Scheme 5. Gratifyingly, the glycosylation of pico donor **19** with 4-OH glucosamine acceptor **6** in the presence of NIS and TfOH in 1,2-dichloroethane as solvent at −30 to 0 °C delivered the desired disaccharide **20** in 1:8.4 α/β selectivity and 80% yield. The minor unwanted α-isomer could be cleanly separated by flash silica gel column chromatography using a EtOAc/hexane solvent mixture to obtain the desired β isomer in pure form. While this work was in progress, Seeberger and co-workers

Scheme 5. Synthesis of Disaccharide Acceptor 3

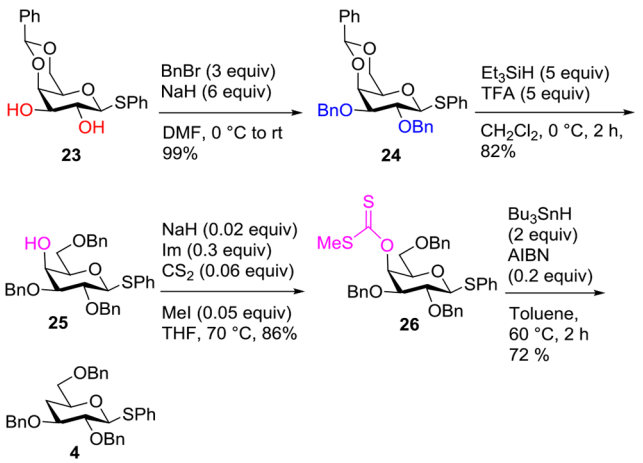


reported a moderate 1:3 α/β selectivity with a less reactive 4-OH glucosyl acceptor using a 3-*O*-picoloyl donor.[15]

Depicolylation of disaccharide **20** in the presence of $Cu(OAc)_2$ in MeOH and CH_2Cl_2[16] cleanly afforded disaccharide 3′-OH derivative **21** in 80% yield. Benzoylation of the free 3′-OH using BzCl and pyridine afforded disaccharide **22** in 82% yield. $PdCl_2$-catalyzed deallylation of disaccharide **22** cleanly furnished disaccharide 2′-OH acceptor **3** in 78% yield. The spectral data of **3** were identical to the data of previously obtained compound **3** synthesized via a C2′ inversion route (Scheme 3). Disaccharide **21** constitutes the backbone disaccharide repeating unit of *P. fluorescens* BIM B-582.

For the synthesis of the rare 4-deoxy sugar (Scheme 6), the known 2,3-diol **23**[17] was benzylated using BnBr and NaH to

Scheme 6. Synthesis of 4-Deoxythio Donor 4

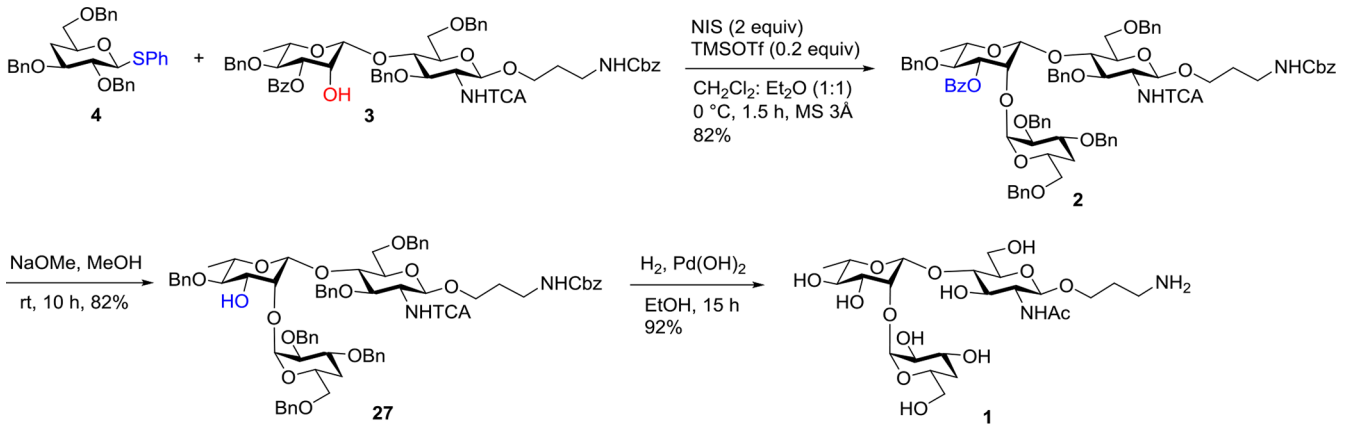


afford 2,3-di-*O*-benzyl derivative **24** in 99% yield. Reductive benzylidene ring opening of **24** using TFA and Et_3SiH furnished 4-OH thiogalactoside donor **25** (82%), which upon treatment with CS_2, MeI, and NaH[18] afforded xanthate ester **26** (71%). The so-formed xanthate was deoxygenated by using TBTH and AIBN to deliver 4-deoxy thio donor **4** in 72% yield.

Assembly of the target molecule, its functional group transformation, and global deprotection are delineated in Scheme 7. The 4-deoxy thioglycoside donor **4** was activated by NIS and TMSOTf promoter in CH_2Cl_2/Et_2O as the participating solvent and glycosylated with acceptor **3** to cleanly

DOI: 10.1021/acs.orglett.8b02669
Org. Lett. XXXX, XXX, XXX−XXX

Scheme 7. Assembly of Trisaccharide 2 and Global Deprotection



afford the desired α-linked trisaccharide **2** as a single isomer in 82% yield. Selective removal of the benzoyl group in the presence of NaOMe and MeOH furnished trisaccharide alcohol **27** in 82% yield. Hydrogenolytic removal of the benzyl groups and concomitant reduction of NHTCA to NHAc using H_2/$Pd(OH)_2$ and filtration furnished the target trisaccharide **1** in 92% yield.

In conclusion, we have accomplished the first total synthesis of the trisaccharide repeating unit of the *O*-specific polysaccharide from *P. fluorescens* BIM B-582. The glycan is equipped with a β-*O*-linked aminopropyl linker at the reducing end to allow for further attachment to carrier proteins. The key features of the synthesis are efficient synthesis of the challenging β rhamnosidic disaccharide via double inversion or HAD mediated direct glycosylation pathways and its further elaboration into the target trisaccharide via 1,2-*cis* glycosylation of a rare 4-deoxy sugar. Trisacccharide **1**, which may play immunodominant roles, is now available for bioevaluation to assess its immunological potential.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02669.

Experimental details and procedures, compound characterization data, and copies of ^{1}H and ^{13}C spectra for all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

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

ORCID

Suvarn S. Kulkarni: 0000-0003-2884-876X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the Science and Engineering Research Board, DST (Grant No. EMR/2014/000235) and the ISF-UGC Joint Research Program Framework (Grant No. 2253). A.B. thanks UGC−New Delhi and IIT Bombay for research fellowships. D.R. and D.K. thank DST for an Inspire Fellowship and National Post Doctoral Fellowship, respectively.

■ REFERENCES

(1) Sperandio, D.; Rossignol, G.; Guerillon, J.; Connil, N.; Orange, N.; Feuilloley, M. G.; Merieau, A. *BMC Microbiol.* **2010**, *10*, 124.

(2) (a) Dogan, B.; Boor, K. J. *Appl. Environ. Microbiol.* **2003**, *69*, 130−138. (b) Del Olmo, A.; Calzada, J.; Nunez, M. *Food Control* **2018**, *86*, 359−366.

(3) Delaquis, P. J.; McCurdy, A. R. *J. Food Sci.* **1990**, *55*, 898−902.

(4) O'Toole, G.; Kaplan, H. B.; Kolter, R. *Annu. Rev. Microbiol.* **2000**, *54*, 49−79.

(5) Sillankorva, S.; Oliveira, R.; Vieira, M. J.; Sutherland, I.; Azeredo, J. *FEMS Microbiol. Lett.* **2004**, *241*, 13−20.

(6) Sillankorva, S.; Neubauer, P.; Azeredo, J. *BMC Biotechnol.* **2008**, *8*, 79.

(7) Valueva, O. A.; Rakhuba, D.; Shashkov, A. S.; Zdorovenko, E. L.; Kiseleva, E.; Novik, G.; Knirel, Y. A. *J. Nat. Prod.* **2011**, *74*, 2161−2167.

(8) For β-rhamnosylation, see: (a) El Ashry, E. S. H.; Rashed, N.; Ibrahim, E. S. I. *Tetrahedron* **2008**, *64*, 10631−10648. (b) Lee, Y. J.; Ishiwata, A.; Ito, Y. *J. Am. Chem. Soc.* **2008**, *130*, 6330−6331. (c) Crich, D.; Li, L. *J. Org. Chem.* **2009**, *74*, 773−781. (d) Yasomanee, J. P.; Demchenko, A. V. *J. Am. Chem. Soc.* **2012**, *134*, 20097−20102. (e) Heuckendorff, M.; Pedersen, C. M.; Bols, M. *J. Org. Chem.* **2012**, *77*, 5559−5568. (f) Zhu, Y.; Shen, Z.; Li, W.; Yu, B. *Org. Biomol. Chem.* **2016**, *14*, 1536−1539.

(9) (a) Crich, D.; Sun, S. *J. Org. Chem.* **1996**, *61*, 4506−4507. (b) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435−436. (c) Kim, K. S.; Kim, J. H.; Lee, Y. J.; Lee, Y. J.; Park, J. *J. Am. Chem. Soc.* **2001**, *123*, 8477−8481. (d) Baek, J. Y.; Choi, T. J.; Jeon, H. B.; Kim, K. S. *Angew. Chem., Int. Ed.* **2006**, *45*, 7436−7440.

(10) Emmadi, M.; Kulkarni, S. S. *J. Org. Chem.* **2011**, *76*, 4703−4709.

(11) Sanapala, S. R.; Kulkarni, S. S. *Org. Lett.* **2016**, *18*, 3790−3793.

(12) Sanapala, S. S.; Kulkarni, S. S. *J. Am. Chem. Soc.* **2016**, *138*, 4938−4947.

(13) Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A. *Org. Lett.* **2014**, *16*, 4838−4841.

(14) Bourke, J.; Brereton, C. F.; Gordon, S. V.; Lavelle, E.; Scanlan, E. M. *Org. Biomol. Chem.* **2014**, *12*, 1114−1123.

(15) Emmadi, M.; Khan, N.; Lykke, N.; Reppe, K.; Parameswarappa, S. G.; Lisboa, M. P.; Wienhold, M. P.; Witzenrath, M.; Pereira, C. L.; Seeberger, P. H. *J. Am. Chem. Soc.* **2017**, *139*, 14783−14791.

(16) Kayastha, A. K.; Jia, X. G.; Yasomanee, J. P.; Demchenko, A. V. *Org. Lett.* **2015**, *17*, 4448−4451.

(17) Podilapu, A. R.; Kulkarni, S. S. *Org. Lett.* **2017**, *19*, 5466−5469.

(18) Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574−1585.

DOI: 10.1021/acs.orglett.8b02669
Org. Lett. XXXX, XXX, XXX−XXX