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Automated Solution-Phase Synthesis of β-1,4-Mannuronate and β-1,4-Mannan

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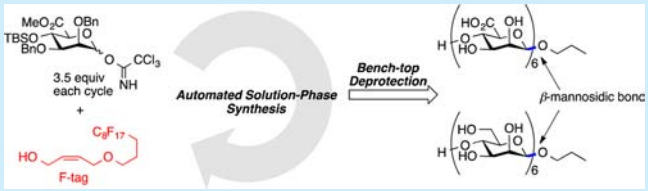
Supporting Information

ABSTRACT: The first automated solution-phase synthesis of β-1,4-mannuronate and β-1,4-mannan oligomers has been accomplished by using a β-directing C-5 carboxylate strategy. By utilizing fluorous-tag assisting purification after repeated reaction cycles, β-1,4-mannuronate was synthesized up to a hexasaccharide with limited loading of a glycosyl donor (up to 3.5 equiv) for each glycosylation cycle due to the homogeneous solution-phase reaction condition. After a global reduction of the uronates, the β-1,4-mannan hexasaccharide was obtained, thereby demonstrating a new approach to β-mannan synthesis.



The β-mannosidic linkage is considered one of the most challenging glycosidic linkages to construct. Both steric hindrance from the 1,2-*cis* configuration and thermodynamic instability from the anomeric effect render the β-anomer less favorable than the α-anomer.[1] However, this linkage is crucial to a variety of natural oligosaccharides, including *N*-linked glycans,[2] antigenic bacterial glycans,[3] immunogenic fungal cell wall mannans,[4] and antifreeze xylomannan.[5] In addition, a variety of natural polysaccharides, such as alginates, contain structurally related β-mannuronic acid. Small oligomers of β-1,4-mannuronic acid have been found to have immunostimulatory properties by activation of the Toll-like receptors (TLR) 2 and 4 and induction of cytokine production.[6] Structurally well-defined synthetic alginate fragments can therefore be potential therapeutic agents and useful tools to study the mechanism of TLR-mediated cytokine production. Other examples of the β-mannosidic linkage in plants are hemicellulose glucomannan and ivory nut mannan; both contain β-1,4 linked mannoses.[7] Herein we report the first strategy for the automated solution-phase synthesis of this class of β-linked oligosaccharides and demonstrate the value of mannuronate building blocks as a precursor donor for stereodefined β-mannose linkages.

A well-known strategy for the synthesis of the β-mannosidic linkages has been developed by Crich and co-workers that uses a conformationally constrained 4,6-*O*-benzylidene-protected thiomannoside building block to provide high β-selectivity during the glycosylation.[8] In fact, this approach was successfully implemented using automated solid-phase synthesis to incorporate up to two β-mannosidic linkages.[9] After formation of the β-mannosidic bond, a series of protecting group shuffles, including removal of the 4,6-*O*-benzylidene acetal under acidic conditions and formation of the ester protecting group at C-6, must be carried out to provide a free OH-4 as an acceptor for further glycosylation. β-1,4-Mannan oligomers up to a hexasaccharide were synthesized manually by this glycosylation−deprotection−esterification sequence.[10] However, a strategy that avoids the acidic deprotection conditions that can also cleave particularly acid-sensitive glycosidic bonds and avoids the extra protection step could potentially shorten a synthetic route to β-1,4-mannan, make it more general, and render it more amenable to the automated synthesis of structures such as the β-mannans and β-1,4-mannuronate oligomers that contain more than two such challenging linkages.

To this end, we were inspired by the elegant work of van der Marel and co-workers in their construction of the β-mannosidic linkages with excellent β-selectivity in the presence of a C-5 carboxylate. After the thio-mannuronate donor was activated by diphenyl sulfoxide and triflic anhydride, the formed anomeric α-triflate, which is stabilized by the C-5 electron-withdrawing carboxylate, gave the β-anomer via an S_N2 mechanism.[11] Also, this group proposed that the relatively stable $^3H_4$ half-chair oxocarbenium intermediate could give rise to high β-selectivity in an S_N1-like manner by attack of the nucleophile from the β-face along a pseudoaxial trajectory. The stability of the $^3H_4$ half-chair oxocarbenium intermediate comes from through-space stabilization of the cation by the pseudoaxial C-5 carboxylate and the most favorable position of other ring substituents.[11d,e] By using this strategy, the β-1,4-mannuronate oligomers up to a pentasaccharide were successfully synthesized.[11b] We reasoned that, if conditions could be found for the global reduction of the uronates, the use of mannuronate building blocks could offer a unique synthetic strategy to the β-mannosidic linkages and one that was also amenable to automation (Scheme 1). Of course, any strategy amenable to automation is practically limited to

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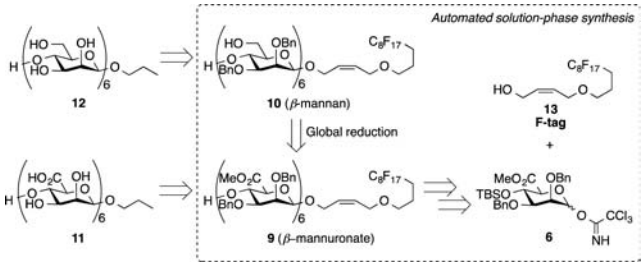
Scheme 1. Retrosynthetic Strategy

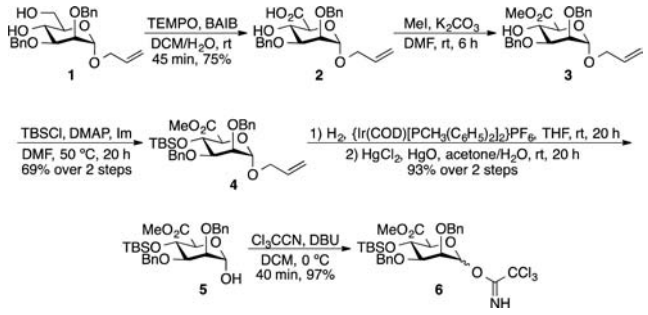


reagents, reactants, and intermediates that are easily dispensed by a liquid handling system and that do not react with the automated system components.

Demonstration of a protocol under automated conditions is of particular importance if this difficult linkage is to be readily incorporated in the design of carbohydrate libraries. Previously, in order to adapt to an automated solid-phase synthesis approach, the 4,6-*O*-benzylidene-carboxybenzylmannosyl donors were used for the "non-pre-activation" protocol under relatively higher temperature (−30 °C) compared to the −78 °C to −60 °C needed for desired selectivities with mannosyl sulfoxide donors.[9a] A less bulky but costlier (triisopropylsiloxy)methyl (Tom) protecting group was employed at the OH-3 position in order to provide higher β-selectivity compared to the bulkier *tert*-butyldimethylsilyl (TBS) group.[9a] In addition, a large excess of donors (9.0 to 10 equiv per coupling cycle) had to be used.[9a] Even for the recently reported automated solid-phase synthesis of the β-mannuronic acid alginates, up to 9.0 equiv of donor was charged to the synthesizer. Although 20% of unreacted donor was claimed to be recovered after glycosylation, only 11% of the donor could be converted to the desired glycoside, and 69% (6.2 equiv) of donor was sacrificed.[9d] In contrast, our automated solution-phase synthesis platform for oligosaccharide synthesis provides a more efficient way to synthesize oligosaccharides in which benchtop protocols can be readily adapted to automation.[12] We discovered that attachment of a C_8F_{17} fluorous-tag (F-tag, **13**) to an initial sugar building block provided a strong noncovalent interaction for the reliable and standardized automated purification by fluorous solid phase extraction (FSPE) of a variety of growing sugar chains.[12,13] All reactions with the fluorous-modified sugars are homogeneous in solution and can be easily monitored off-line by thin layer chromatography (TLC) of automatically sampled reaction aliquots. Because of the homogeneous reaction environment, significantly lower amounts (1.5 to 3.5 equiv) of glycosyl donors are needed for the glycosylation compared to a heterogeneous solid-phase approach.[12]

To implement an approach to the β-mannosidic linkages via mannuronates, a suitably protected building block had to be designed. Of particular importance was the choice of masking group for the OH-4, as it has to be reliably removed for chain extension. The TBS group was chosen as the temporary protecting group on OH-4 for our automated synthesis due to its electron-donating nature, which confers higher reactivity on the glycosylation compared to the electron-withdrawing levulinoate group.[14] The synthesis of the desired mannuronate building block **6** started from the previously reported allyl mannoside **1**[15] (Scheme 2), which was oxidized to the mannuronic acid **2** by 2,2,6,6-tetramethyl-1-piperidinyloxy free radical (TEMPO)/(diacetoxyiodo)benzene (BAIB) fol-

Scheme 2. Synthesis of the Building Block 6 for the Automated Solution-Phase Synthesis of β-1,4-Mannuronate and β-1,4-Mannan Hexamers



lowed by an esterification with methyl iodide/K_2CO_3 to give the methyl mannuronate **3**.[11a] Silylation of **3** with *tert*-butyldimethyl silyl chloride (TBSCl)/imidazole/4-dimethylaminopyridine (DMAP) gave the fully protected mannuronate **4**. The subsequent combination of hydrogen gas and a catalytic amount of (1,5-cyclooctadiene)bis(methyldiphenylphosphine)-iridium(I) hexafluorophosphate, followed by the $HgCl_2$/HgO condition, removed the allyl group to afford **5** with a free anomeric OH. The installation of the trichloroacetimidate at the OH-1 position furnished the building block **6** for the automated solution-phase synthesis (Scheme 2).

The synthesis of the β-1,4-mannuronate hexamer **9** and the β-1,4-mannan hexamer **10** in the automated synthesis platform (Scheme 3) began with attachment of the F-tag **13** to the building block **6**. After the platform transferring solution of **13** and **6** (3.0 equiv) from the stock solution station to the reactor, the temperature control unit lowered the temperature to −20 °C and trimethylsilyl trifluoromethanesulfonate (TMSOTf) stock solution (0.1 equiv) was added to start the reaction. After 30 min, a small aliquot of the reaction mixture was automatically collected for later off-line thin layer chromatography (TLC) analysis. The reaction was then quenched by triethylamine (TEA), and the solvent was removed under reduced pressure generated by the vacuum pump unit at elevated temperature. The deprotection was carried on without further purification. Various conditions were tested to remove the TBS group without degrading the esters and glycosidic linkages (Table S4). A particular limitation of any reagent used is that the chemical and its byproducts do not etch the precision glass reactors. Tetrabutylammonium fluoride (TBAF)/tetrahydrofuran (THF) was found to be too basic for the methyl ester; however, the buffered conditions (TBAF/AcOH) turned out to lack reactivity.[11e] The milder HF-pyridine/pyridine condition was eliminated due to its glass-etching property. Triethylamine trihydrofluoride (TEA·3HF) did not corrode the glassware but was found to be too acidic for maintenance of the glycosidic linkages. Finally, a mixture of TBAF and TEA·3HF (molar ratio = 4/1) provided a mild condition with enough strength for desilylation while also being safe for the glass reactor. After TLC monitoring showed the completion of deprotection, the solvent was removed under reduced pressure and the mixture was loaded onto the FSPE cartridge. The automated synthesis platform performed elution, product fractions collection, and solvent evaporation and let the redissolved product pass through a silica cartridge SPE for removal of additional impurities. The crude product was transferred out of the platform and further purified to obtain the monosaccharide **7** (74% over 2 steps). A particular

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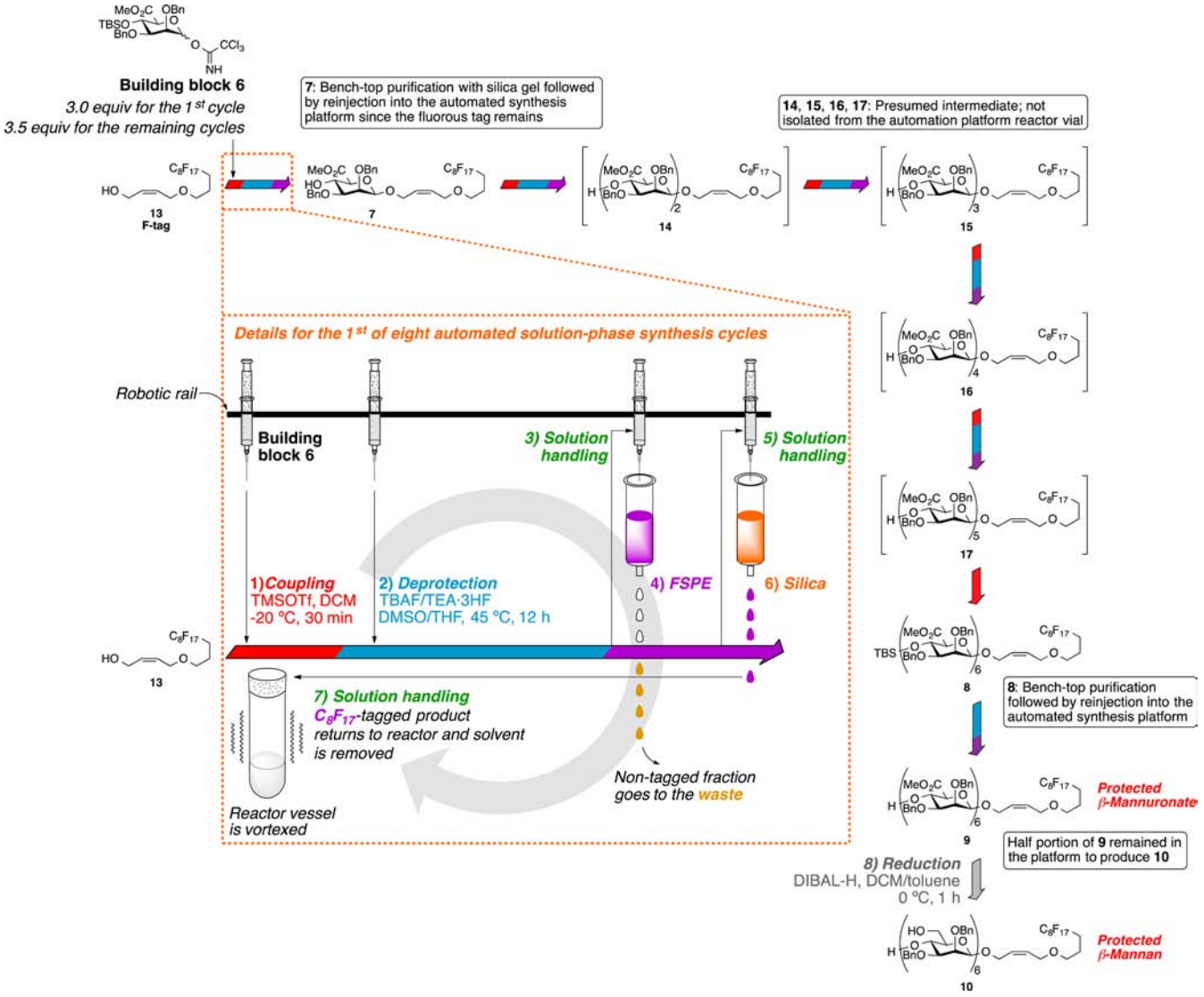
Scheme 3. Automated Solution-Phase Synthesis of β-1,4-Mannuronate and β-1,4-Mannan Hexamers

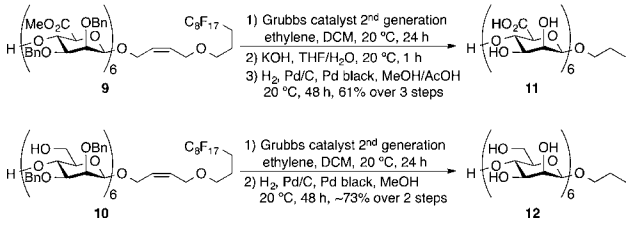


advantage of this solution-phase approach over solid-phase approaches is exactly this ability to easily carry out additional purification steps if needed prior to the end of the entire synthesis sequence. After **7** (50 μmol) was reinjected to the automated synthesis platform and followed by four repeating glycosylation−deprotection cycles plus one more glycosylation utilizing 3.5 equiv of **6** for each glycosylation step, the product mixture was purified on benchtop to afford the fully protected hexamannuronate **8** in 7% yield over 9 steps (75% average per reaction step).

The hexasaccharide **8** was reinjected to the automated synthesis platform followed by a deprotection cycle to produce compound **9**. A half portion of **9** was retained in the platform and treated with diisobutylaluminum hydride (DIBAL-H) and stirred for 1 h at 0 °C for the global reduction of the esters into alcohols to afford the β-1,4-mannan hexamer **10** in 82% yield over 2 steps (Scheme 3). The yield of the overall automated solution-phase synthesis scheme is influenced by the liquid handling ability of the automated synthesis platform since it was difficult to achieve absolutely quantitative liquid transfers between the reactor blocks and the FSPE blocks.

Bench-top deprotection of the β-mannuronate hexamer **9** started with cleavage of the fluorous tag by olefin cross-metathesis, followed by hydrolysis of the esters and hydrogenolysis of the benzyl ethers to afford the fully deprotected β-1,4-mannuronate hexamer **11** in 61% yield over 3 steps. The fluorous tag of **10** was cleaved by olefin cross-metathesis as well and followed by hydrogenolysis of the benzyl groups, which provided the fully deprotected β-1,4-mannan hexamer **12** in around 73% yield over 2 steps (Scheme 4).

Scheme 4. Deprotection of β-1,4-Mannuronate Hexamer 9 and β-1,4-Mannan Hexamer 10



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In conclusion, we report a strategy for the first automated solution-phase synthesis of the β-1,4-mannuronate and β-1,4-mannan oligomers. By using significantly limited amounts of the glycosyl donor per glycosylation cycle, a 4-OTBS protecting group with mild desilylation condition, fluorous-tag-assisted purification, and real-time reaction monitoring, automated synthesis of oligosaccharides up to hexamers has been achieved. Also, global reduction of methyl β-mannuronates proved to be effective to synthesize β-mannans. We are currently exploring the scope of this strategy for the synthesis of other β-mannosidic-linkage-containing natural products, and its capability to be adapted to other automated synthesis platforms.[16]

■ ASSOCIATED CONTENT

Supporting Information

Automated solution-phase synthesis protocols, synthetic procedures, and spectral data. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01013.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Heuckendorff, M.; Bendix, J.; Pedersen, C. M.; Bols, M. *Org. Lett.* **2014**, *16*, 1116−1119. (b) Baek, J. Y.; Lee, B.-Y.; Jo, M. G.; Kim, K. S. *J. Am. Chem. Soc.* **2009**, *131*, 17705−17713. (c) Stork, G.; La Clair, J. J. *J. Am. Chem. Soc.* **1996**, *118*, 247−248. (d) Wu, X.; Bundle, D. R. *J. Org. Chem.* **2005**, *70*, 7381−7388.

(2) (a) Gamblin, D. P.; Scanlan, E. M.; Davis, B. G. *Chem. Rev.* **2009**, *109*, 131−163. (b) Schmaltz, R. M.; Hanson, S. R.; Wong, C.-H. *Chem. Rev.* **2011**, *111*, 4259−4307.

(3) (a) Crich, D.; Li, H.; Yao, Q.; Wink, D. J.; Sommer, R. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2001**, *123*, 5826−5828. (b) Crich, D.; Banerjee, A.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 14930−14934.

(4) (a) Nitz, M.; Purse, B. W.; Bundle, D. R. *Org. Lett.* **2000**, *2*, 2939−2942. (b) Nitz, M.; Bundle, D. R. *J. Org. Chem.* **2001**, *66*, 8411−8423. (c) Wu, X.; Bundle, D. R. *J. Org. Chem.* **2005**, *70*, 7381−7388.

(5) (a) Walters, K. R., Jr.; Serianni, A. S.; Sformo, T.; Barnes, B. M.; Duman, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20210−20215. (b) Ishiwata, A.; Sakurai, A.; Nishimiya, Y.; Tsuda, S.; Ito, Y. *J. Am. Chem. Soc.* **2011**, *133*, 19524−19535. (c) Crich, D.; Rahaman, M. Y. *J. Org. Chem.* **2011**, *76*, 8611−8620.

(6) Iwamoto, M.; Kurachi, M.; Nakashima, T.; Kim, D.; Yamaguchi, K.; Oda, T.; Iwamoto, Y.; Muramatsu, T. *FEBS Lett.* **2005**, *579*, 4423−4429.

(7) Robyt, J. F. *Essentials of Carbohydrate Chemistry*; Springer: New York, 1998.

(8) (a) Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144−1153. (b) Crich, D. *J. Org. Chem.* **2011**, *76*, 9193−9209. (c) Weingart, R.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41*, 8753−8758.

(9) (a) Codee, J. D. C.; Kroeck, L.; Castagner, B.; Seeberger, P. H. *Chem.—Eur. J.* **2008**, *14*, 3987−3994. (b) Seeberger, P. H. *Chem. Soc. Rev.* **2008**, *37*, 19−28. (c) Hsu, C.-H.; Hung, S.-C.; Wu, C.-Y.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2011**, *50*, 11872−11923. (d) Walvoort, M. T. C.; van den Elst, H.; Plante, O. J.; Kröck, L.; Seeberger, P. H.; Overkleeft, H. S.; van der Marel, G. A.; Codée, J. D. C. *Angew. Chem., Int. Ed.* **2012**, *51*, 4393−4396.

(10) (a) Crich, D.; Banerjee, A.; Yao, Q. *J. Am. Chem. Soc.* **2004**, *126*, 14930−14934. (b) Kim, K. S.; Baburao Fulse, D.; Baek, J. Y.; Lee, B.-Y.; Jeon, H. B. *J. Am. Chem. Soc.* **2008**, *130*, 8537−8547.

(11) (a) van den Bos, L. J.; Dinkelaar, J.; Overkleeft, H. S.; van der Marel, G. A. *J. Am. Chem. Soc.* **2006**, *128*, 13066−13067. (b) Codee, J. D. C.; van den Bos, L. J.; de Jong, A.-R.; Dinkelaar, J.; Lodder, G.; Overkleeft, H. S.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 38−47. (c) Dinkelaar, J.; de Jong, A. R.; van Meer, R.; Somers, M.; Lodder, G.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2009**, *74*, 4982−4991. (d) Walvoort, M. T. C.; Lodder, G.; Mazurek, J.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. *J. Am. Chem. Soc.* **2009**, *131*, 12080−12081. (e) Walvoort, M. T. C.; Moggre, G.-J.; Lodder, G.; Overkleeft, H. S.; Codee, J. D. C.; van der Marel, G. A. *J. Org. Chem.* **2011**, *76*, 7301−7315.

(12) Pohl, N. L. In *Chemical Glycobiology*; Chen, X., Halcomb, R., Wang, G. P., Eds.; ACS Symposium Series 990; American Chemical Society: Washington, DC, 2008; pp 272−287.

(13) (a) Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162−13163. (b) Mamidyala, S. K.; Ko, K.-S.; Jaipuri, F. A.; Park, G.; Pohl, N. L. *J. Fluorine Chem.* **2006**, *127*, 571−579. (c) Liu, L.; Pohl, N. L. B. *Org. Lett.* **2011**, *13*, 1824−1827. (d) Lin, L.; Pohl, N. L. B. *Carbohydr. Res.* **2013**, *369*, 14−24.

(14) Zeng, Y.; Wang, Z.; Whitfield, D.; Huang, X. *J. Org. Chem.* **2008**, *73*, 7952−7962.

(15) Srivastav, S. S.; Khare, N. K.; Khare, A. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **2000**, *39B*, 346−351.

(16) Ganesh, N. V.; Fujikawa, K.; Tan, Y. H.; Stine, K. J.; Demchenko, A. V. *Org. Lett.* **2012**, *14*, 3036−3039.

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