Organic LETTERS

Letter

pubs.acs.org/OrgLett

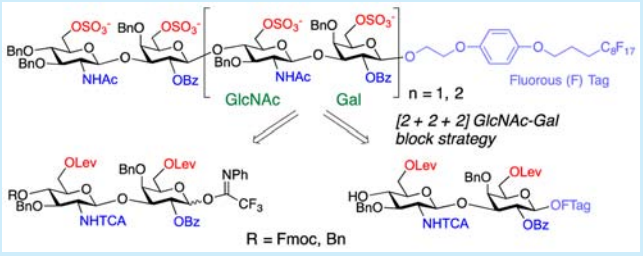
Fluorous-Tag Assisted Syntheses of Sulfated Keratan Sulfate Oligosaccharide Fragments

Sayantan Bhaduri and Nicola L. B. Pohl*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States

Supporting Information

ABSTRACT: The first block iteration strategy for iterative solution-phase synthesis of protected keratan sulfate (KS)-like fragments is reported. Obstacles in a strategy using galactose-glucosamine (Gal-GlcNAc) modules led to the discovery of a differentially protected GlcNAc-Gal module that could be used to synthesize KS-like fragments using a fluorous tag that maintained solubility in organic solvents for purification of all intermediates via fluorous solid-phase extraction.



Keratan sulfate (KS), one of the major subclasses of the glycosaminoglycan (GAG) family, has at its core a linear, sulfated oligosaccharide consisting of *N*-acetyl-β-D-glucosamine (GlcNAc) and β-D-galactose (Gal) residues linked together via alternative 1 → 3 and 1 → 4 linkages (Figure 1).[1] KS is structurally unique compared to other GAGs, such as the well-studied heparin/heparan sulfates,[2] in its absence of any acidic residue (uronic acid), which is replaced with a neutral D-galactose moiety, and the presence of variable capping carbohydrate residues at the nonreducing terminal of the KS chain.[1] Despite its unique structural features and myriad important biological roles,[1,3] KS has attracted surprisingly little attention from the synthetic community. To date, synthetic efforts have mostly focused only on the chemical synthesis of related subclasses of GAGs: heparin/heparan sulfate,[4] chondroitin sulfate,[5] dermatan sulfate,[6] and hyaluronan.[7] In contrast, syntheses of a few KS di- and trisaccharide fragments[8] and only two partially sulfated KS tetrasaccharide fragments[9] have been reported. This lack of methods to make KS is surprising given that naturally sourced KS is so heterogeneous as to hamper molecular studies.[10] The nonreducing terminus of the corneal KS chain with its highly sulfated backbone **1** provides a good synthetic target for developing a strategy to KS synthesis (Figure 1). However, the installation of multiple sulfate groups on its backbone imposes greater complexities in the orthogonal protecting group manipulations for the synthesis of higher order analogues beyond di- and trisaccharides. In addition, synthesis of larger KS fragments demands an appropriate endgame involving removal of orthogonal protecting groups, purification of small quantities of highly polar molecules, and most importantly, multiple sulfate group installations in the presence of highly deactivating groups such as *N*-acetyl (NHAc) and esters which are mandatory to synthesize β-linkages present in KS-like structures.

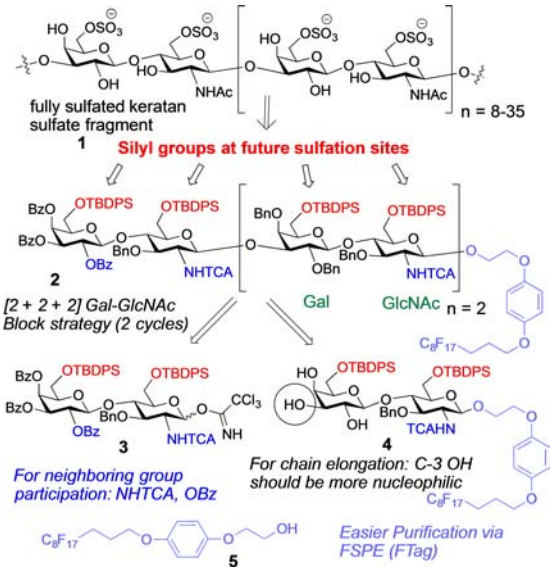


Figure 1. Retrosynthetic strategy for the KS chain using a Gal-GlcNAc disaccharide module.

The pioneering synthesis of a trisulfated tetrasaccharide by Ogawa and co-workers relied on a sequential glycosylation strategy that results in a low-yielding glycosylation reaction (48%) to synthesize the final tetrasaccharide using a [1 + 3] coupling plan and multiple protecting group manipulations at the di- and trisaccharide stage to install sulfate groups.[9] In the other chemoenzymatic strategy, glycosylation yields were affected by the hydrolysis of disaccharide precursor under the enzymatic reaction conditions.[9] These prior syntheses demonstrate the inherent difficulties associated with the synthesis of longer KS chains, since successful methods to make smaller sugars often do not directly translate into a viable strategy for production of larger oligosaccharides. Herein, we report the first block iteration strategy using a fluorous tag amenable to automation in which

Received: February 2, 2016
Published: March 9, 2016

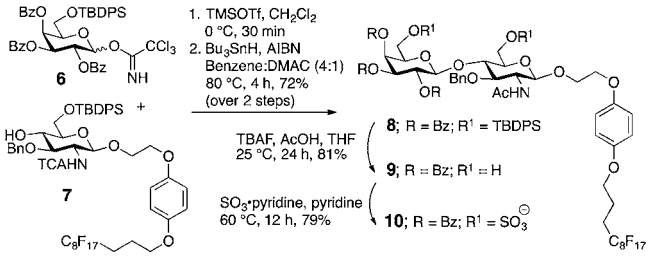
DOI: 10.1021/acs.orglett.6b00344
Org. Lett. 2016, 18, 1414−1417

rapid assembly of the KS chain is made possible by avoiding multiple glycosylation/deprotection sequences required by the prior sequential strategy[9] and is not limited to the creation of fragments smaller than a tetrasaccharide.

The repeating nature of the KS backbone lends itself to a [2 + 2] block approach, especially when fragments longer than a tetrasaccharide are needed. Retrosynthesis leads to two possible modules: (1) Gal-GlcNAc and (2) GlcNAc-Gal. The initial strategy then aimed to make a Gal-GlcNAc module (Figure 1) with a fluorous tag at the reducing end for easier purification of all intermediates via fluorous solid-phase extraction (FSPE)[11] and ultimately automation.[12] The building blocks were designed with *tert*-butyldiphenylsilyl (TBDPS) groups[9,13] for later installation of sulfates plus benzoyl (Bz) and *N*-trichloroacetamide (NHTCA) groups[5,14] at the C-2 positions for anchimeric assistance in forming the required β-linkages. Benzyl (Bn) groups were chosen to mask the remaining hydroxyls.[15] Fluorous tag **5** (Figure 1) was chosen over an allyl-based C_8F_{17} tag[16] to increase flexibility in reagent choice for glycosylation reactions and thereby glycosyl donor choice. TBDPS was chosen in preference over other commonly used ether groups such as TBS (*tert*-butyldimethylsilyl), TIPS (triisopropylsilyl), and PMB (4-methoxybenzyl), and NHTCA was chosen over other amine protecting groups such as 2,2,2-trichloroethyl chloroformate (Troc)[17] and phthalic anhydride (Phth)[18] owing to their stability under both acidic and basic conditions. The *N*-Phth and *N*-Troc groups have proven incompatible with base-catalyzed reactions.[19]

The chemical synthesis of the smallest KS repeating unit, disaccharide fragment **10**, was first attempted (Scheme 1) to test

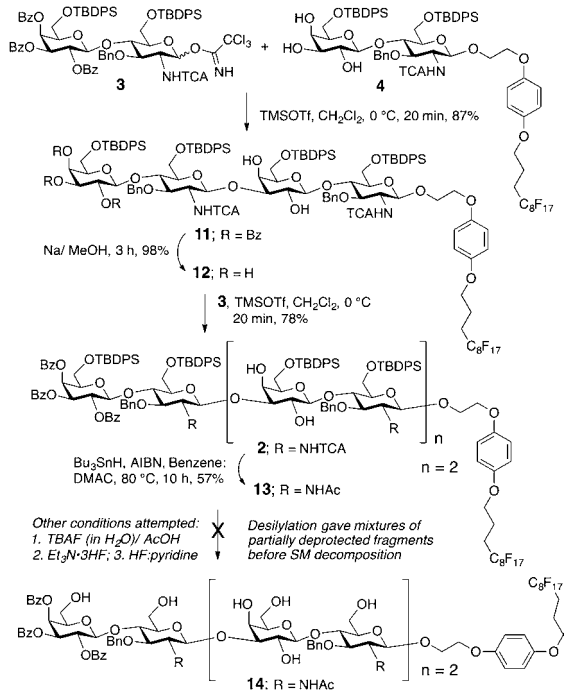
Scheme 1. Synthesis of KS Disaccharide Fragment 10



the reactivity of monosaccharide donor **6** and acceptor **7** during glycosylation and to develop orthogonal TBDPS deprotection[9,13] and sulfation conditions (see the Supporting Information). In addition, these highly polar fluorous-tagged molecules were examined for their amenability to purification by FSPE. To this end, the sulfated KS disaccharide fragment **10** was successfully made and fortunately proved amenable to FSPE.

With this initial success in the synthesis of the basic sulfated KS module **10**, the potential for making larger KS fragments via a Gal-GlcNAc modular strategy was probed. The nucleophilicity of the C-3 hydroxyl group of galactose is known to be greater than its other secondary hydroxyls.[20] Therefore, the desired glycosidic bond to achieve longer KS fragments possibly could be formed regioselectively through the C-3 hydroxyl of triol acceptor **4** (Figure 1). Gratifyingly, disaccharide donor **3** and acceptor **4** were successfully coupled to form the desired 1 → 3-linked tetrasaccharide **11** in 87% isolated yield (Scheme 2), as confirmed by 2D NMR analysis (see the Supporting Information). This result shows the viability of this alternative approach for the rapid construction of oligosaccharides with minimal protecting group manipulations compared to the Ogawa study.[9] The C-2′ and C-4′ hydroxyls of compound **11** proved recalcitrant to any

Scheme 2. Successful Syntheses of KS Tetra- and Hexasaccharides 11 and 2 with Gal-GlcNAc Modules but Desilylation Attempts Failed



modification, likely due to steric congestion by multiple nearby TBDPS groups (see the Supporting Information). Further chain extension was then attempted directly with these two sterically hindered hydroxyls unmasked since the substrate was still soluble in CH_2Cl_2. Saponification of the benzoates on tetrasaccharide **11** followed by glycosylation with **3** afforded our desired hexasaccharide **2** in 78% isolated yield. This Gal-GlcNAc strategy is clearly feasible to produce longer protected KS backbone motifs. Initial attempts to convert three NHTCA groups to the naturally occurring NHAc via tributyltin hydride/azobis-(isobutyronitrile) (AIBN)-mediated radical reaction in benzene proved extremely challenging.[21] Thin-layer chromatography (TLC) analysis showed complex mixtures, likely of partially substituted chlorinated products. However, addition of dimethylacetamide (DMAC) as a cosolvent[5] accelerated the progress of the reaction, and compound **13** was obtained as a single product in 57% isolated yield. Furthermore, purification of the product from excess tin byproducts was simple via FSPE.[11] However, the deprotection of six TBDPS groups from the large hexasaccharide **13** led to mixtures of differentially deprotected compounds. Applied heat (35 °C) resulted in decomposition of starting material (SM).

The robustness of the multiple TBDPS groups forced an alternative retrosynthetic strategy using orthogonal ester functionalities to decorate the desired disaccharide building blocks (Figure 2). TBDPS groups were replaced with levulinoyl esters[4] (Lev) for selective removal in the presence of other ester groups. A naphthylmethyl group was chosen to protect the C-3 hydroxyl group of galactose for subsequent chain extension.[22] However, now the crucial glycosylation had to be reassessed with this switch from relatively electron-donating groups to multiple Lev esters along with other deactivating groups such as Bz and NHTCA. A trial glycosylation reaction between the disaccharide donor **16** and acceptor **18** was tested, but it failed to produce the desired tetrasaccharide **19** (Scheme 3). Unfortunately, the

DOI: 10.1021/acs.orglett.6b00344
Org. Lett. 2016, 18, 1414−1417

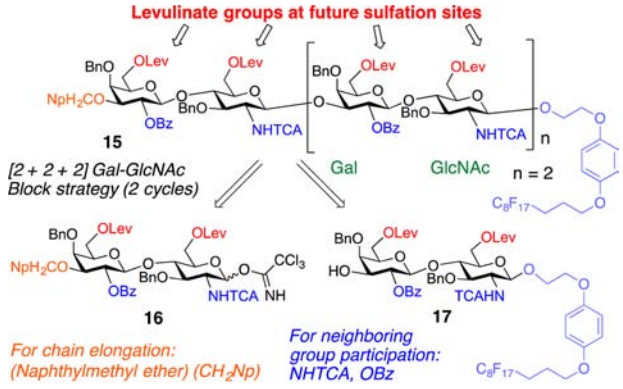
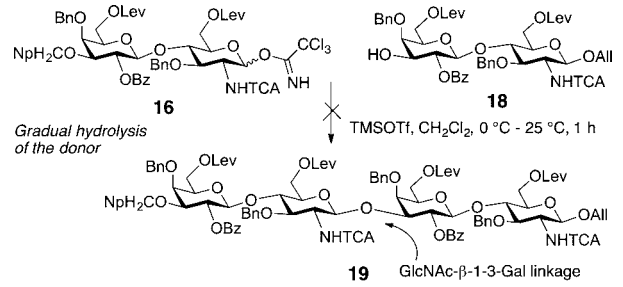


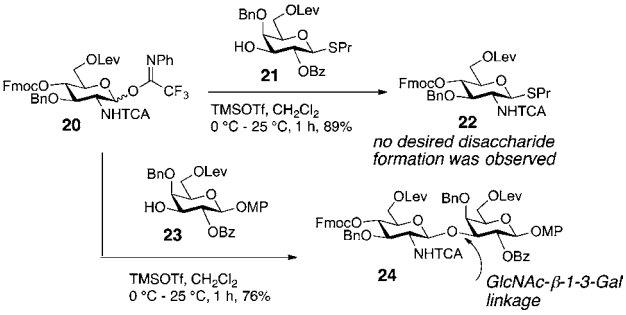
Figure 2. Alternative retrosynthetic strategy for a Gal-GlcNAc module based on orthogonal ester functionalities.

Scheme 3. Failed AttemptTo Construct the Challenging GlcNAc-β-1 → 3-Gal Linkage



multiple ester groups proved to reduce the reactivity of **16** and **18** too much. To test the possibility of remote deactivating effects through glycosidic bonds and not just across glycosidic rings, the reactivity of monosaccharide donor **20** was compared with its disaccharide analogue **16** (Scheme 4). A trial glycosylation

Scheme 4. Successful Construction of the GlcNAc-β-1 → 3-Gal Linkage To Form a New Disaccharide Module



reaction between the monosaccharide donor **20** and acceptor **21** was performed to construct the challenging GlcNAc-β-1 → 3-Gal linkage (Scheme 4). To better match the reduced reactivity of the glycosyl acceptor, the more stable *N*-phenyltrifluoroacetimidate donor[23] **20** rather than a trichloroacetimidate was employed. Surprisingly, intermolecular aglycone transfer **22** was observed as a major isolated product instead of the desired disaccharide formation, which further emphasized the reduced glycosylation power of these building blocks. To attempt to prevent this side reaction, the thiopropyl group (SPr) in acceptor **21** was replaced with the less nucleophilic *p*-methoxyphenyl group (OMP) to make compound **23**. This subtle tuning of the protecting group pattern fortunately did result in the synthesis of the desired disaccharide **24** via TMSOTf-mediated glycosylation (Scheme 4). Given the challenges of forming the GlcNAc-β-1 → 3-Gal linkage, a new strategy using a GlcNAc-Gal module was developed for construction of the final protected KS tetra- and hexasaccharide targets (**25** and **26**, Figure 3) with three

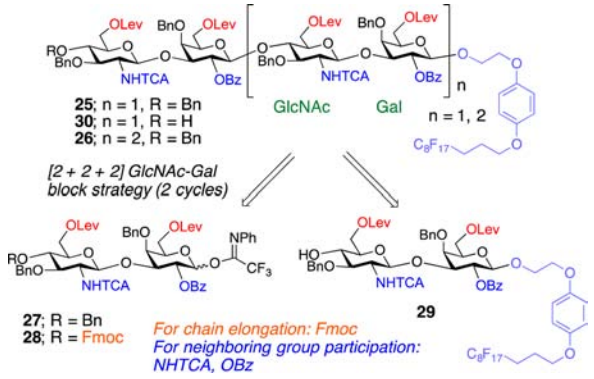
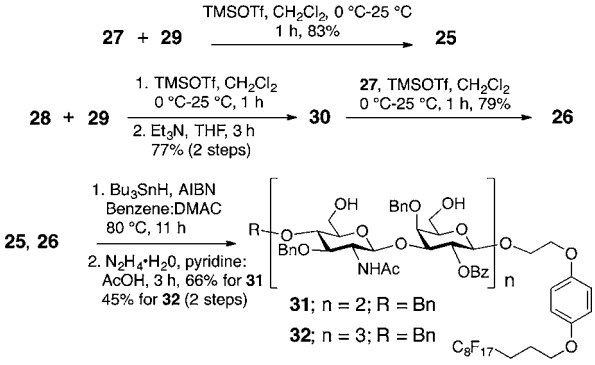


Figure 3. Final strategy for the construction of KS tetra- and hexasaccharide targets **25** and **26** using GlcNAc-Gal modules.

disaccharide building blocks whose syntheses are detailed in the Supporting Information: (1) capping disaccharide donor **27**, (2) repeating disaccharide donor **28** for chain elongation at the C-4′ OH, and (3) disaccharide acceptor **29**. Successful synthesis of tetrasaccharide **25** was achieved via TMSOTf-catalyzed coupling of **27** and **29** in 83% isolated yield (Scheme 5). Similarly, en route

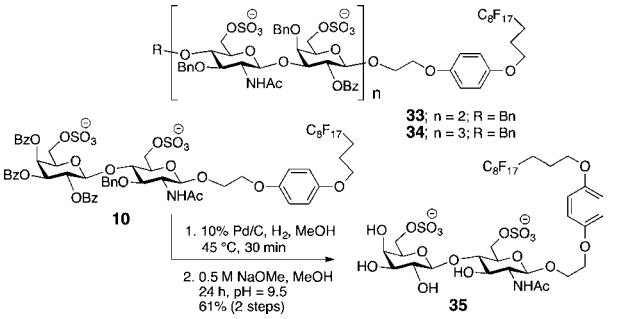
Scheme 5. Successful Syntheses of KS Tetra- And Hexasaccharide 25 and 26



to **26**, coupling of **28** with **29** followed by subsequent removal of the fluorenylmethoxycarbonyl (Fmoc) group[23] offered tetrasaccharide acceptor **30** in 77% yield over two steps. Tetrasaccharide acceptor **30** was further coupled with **27** to afford KS hexasaccharide **26** in 79% yield. Multiple NHTCA groups on **25** and **26** were then transformed to the naturally occurring NHAc groups via radical reactions. Deprotection conditions for multiple Lev esters[4,6] in the presence of benzoyl and NHTCA groups were then examined on these larger fluorous-tagged KS oligosaccharides. Fortunately, multiple Lev esters could be successfully cleaved and the resulting products efficiently purified via FSPE to obtain **31** and **32** (Scheme 5). With the KS backbones in hand, the challenge of multiple sulfation reactions remained, given the lability of sulfates in acidic media,[24] in the presence of several deactivating groups (NHAc and OBz). Screening of several sulfation conditions[4−6,8,9] showed SO_3·NMe_3 in DMF at 55 °C to be best for complete sulfation without side reactions. Tetrasulfated KS fragment **33** (Scheme 6) was thereby

DOI: 10.1021/acs.orglett.6b00344
Org. Lett. 2016, 18, 1414−1417

Scheme 6. Sulfation and Global Deprotection of KS Fragments



successfully synthesized for the first time (17 linear steps, 7% overall yield cf. 21 linear steps, 2% overall yield for the previous report[9]), and this highly charged molecule was isolated in 79% yield as a single product after FSPE purification, thereby bypassing any potential purification issue. Severe peak overlap in the $^{1}H/^{13}C$ NMR spectra prevented definitive assignments; however, four distinct anomeric peaks in the ^{13}C NMR could easily be distinguished, and the lack of protecting groups prone to migration next to the free hydroxyls that were sulfated supports a single tetrasulfated isomer. Clearly, our methodology proves promising for automated fluorous-based synthesis of longer KS chains. The formation of hexasulfated fragment **34** was also confirmed via high-resolution mass spectrometry [HRMS-ESI-TOF (m/z): $[M - 3H]^{3-}$ calcd for $C_{131}H_{138}F_{17}N_3O_{54}S_6^{3-}$ 1043.5336, found 1043.5215]. After successful construction of highly sulfated KS-like fragments, global deprotection conditions[24] were tested on KS disaccharide **10** (Scheme 6). Continuous flow hydrogenation of **10** followed by hydrolysis of the benzoate esters supplied the fully deprotected KS disaccharide **35** in 61% overall yield over two steps.

In summary, the first fluorous-assisted syntheses of fully protected, highly sulfated KS di-, tetra-, and hexasaccharide fragments have been completed via an efficient block iteration strategy with excellent coupling yields while avoiding the lengthy protection/deprotection steps reported previously.[9] The presence of multiple ester and amide moieties required for synthesis of β-linkages reduced the reactivity of the building blocks and thereby made the growing oligosaccharide chain less reactive toward glycosylation and sulfation reactions. The reactivity of glycosyl acceptors and donors was shown to change significantly when part of the larger oligosaccharides rather than of simple monosaccharides, thereby complicating the creation of longer fragments. Clearly, results on smaller fragments do not directly translate into a viable strategy for the synthesis of larger oligosaccharide fragments. Ultimately, a GlcNAc-Gal disaccharide module proved better than the alternative strategy using a Gal-GlcNAc module. The choice of soluble reagents and the success of the fluorous tag in maintaining solubility in organic solvents of all intermediates should also make the approach amenable toward solution-phase automation of KS-like fragments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00344.

Synthesis protocol and characterization of all new compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: npohl@indiana.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

N.P. acknowledges the Joan and Marvin Carmack fund and Indiana University. This work was also supported in part by the U.S. National Institutes of Health (1R01GM090280).

■ REFERENCES

(1) Funderburgh, J. L. *Glycobiology* **2000**, *10*, 951.
(2) Rabenstein, D. L. *Nat. Prod. Rep.* **2002**, *19*, 312.
(3) Pomin, V. H. *Int. J. Biol. Macromol.* **2015**, *72*, 282.
(4) (a) Polat, T.; Wong, C. H. *J. Am. Chem. Soc.* **2007**, *129*, 12795. (b) Arungundram, S.; Al-Mafraji, K.; Asong, J.; Leach, F. E.; Amster, I. J.; Venot, A.; Turnbull, J. E.; Boons, G. J. *J. Am. Chem. Soc.* **2009**, *131*, 17394.
(5) (a) Gama, C. I.; Tully, S. E.; Sotogaku, N.; Clark, P. M.; Rawat, M.; Vaidehi, N.; Goddard, W. A.; Nishi, A.; Hsieh-Wilson, L. C. *Nat. Chem. Biol.* **2006**, *2*, 467. (b) Eller, S.; Collot, M.; Yin, J.; Hahm, H. S.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2013**, *52*, 5858.
(6) Kandasamy, J.; Schuhmacher, F.; Hahm, H. S.; Klein, J. C.; Seeberger, P. H. *Chem. Commun.* **2014**, *50*, 1875.
(7) Lu, X.; Kamat, M. N.; Huang, L.; Huang, X. *J. Org. Chem.* **2009**, *74*, 7608.
(8) (a) Misra, A. K.; Agnihotri, G.; Madhusudan, S. K.; Tiwari, P. *J. Carbohydr. Chem.* **2004**, *23*, 191. (b) Takeda, N.; Tamura, J. I. *Biosci., Biotechnol., Biochem.* **2014**, *78*, 29.
(9) (a) Kobayashi, M.; Yamazaki, F.; Ito, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *201*, 51. (b) Ohmae, M.; Sakaguchi, K.; Kaneto, T.; Fujikawa, S.; Kobayashi, S. *ChemBioChem* **2007**, *8*, 1710.
(10) Weyers, A.; Yang, B.; Solakyildirim, K.; Yee, V.; Li, L.; Zhang, F.; Linhardt, R. J. *FEBS J.* **2013**, *280*, 2285.
(11) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837.
(12) (a) Tang, S. L.; Pohl, N. L. B. *Org. Lett.* **2015**, *17*, 2642. (b) Tang, S. L.; Linz, L.; Bonning, B.; Pohl, N. L. B. *J. Org. Chem.* **2015**, *80*, 10482.
(13) Jaipuri, F. A.; Pohl, N. L. *Org. Biomol. Chem.* **2008**, *6*, 2686.
(14) Werz, D. B.; Adibekian, A.; Seeberger, P. H. *Eur. J. Org. Chem.* **2007**, *2007*, 1976.
(15) Oguri, H.; Hishiyama, S.; Oishi, T.; Hirama, M. *Synlett* **1995**, *1995*, 1252.
(16) Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162.
(17) Gagarinov, I. A.; Fang, T.; Liu, L.; Srivastava, A. D.; Boons, G. J. *Org. Lett.* **2015**, *17*, 928.
(18) Melean, L. G.; Love, K. R.; Seeberger, P. H. *Carbohydr. Res.* **2002**, *337*, 1893.
(19) (a) Costello, C. A.; Kreuzman, A. J.; Zmijewski, M. J. *Tetrahedron Lett.* **1996**, *37*, 7469. (b) Hansen, S. G.; Skrydstrup, T. *Eur. J. Org. Chem.* **2007**, *2007*, 3392.
(20) Koeller, K. M.; Wong, C. H. *Chem. - Eur. J.* **2000**, *6*, 1243.
(21) Barroca-Aubry, N.; Pernet-Poil-Chevrier, A.; Domard, A.; Trombotto, S. *Carbohydr. Res.* **2010**, *345*, 1685.
(22) Boltje, T. J.; Zhong, W.; Park, J.; Wolfert, M. A.; Chen, W.; Boons, G. J. *J. Am. Chem. Soc.* **2012**, *134*, 14255.
(23) Hanashima, S.; Seeberger, P. H. *Chem. - Asian J.* **2007**, *2*, 1447.
(24) Yang, B.; Yoshida, K.; Yin, Z.; Dai, H.; Kavunja, H.; El-Dakdouki, M. H.; Sungsuwan, S.; Dulaney, S. B.; Huang, X. *Angew. Chem., Int. Ed.* **2012**, *51*, 10185.

DOI: 10.1021/acs.orglett.6b00344
Org. Lett. 2016, 18, 1414–1417