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Synthesis of Fluorosugar Reagents for the Construction of Well-Defined Fluoroglycoproteins

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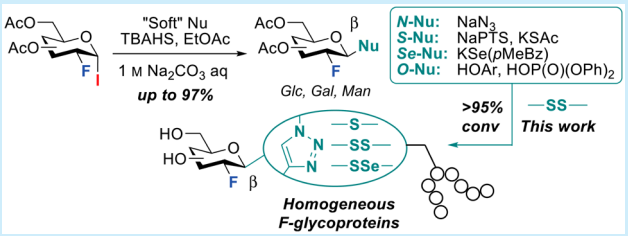
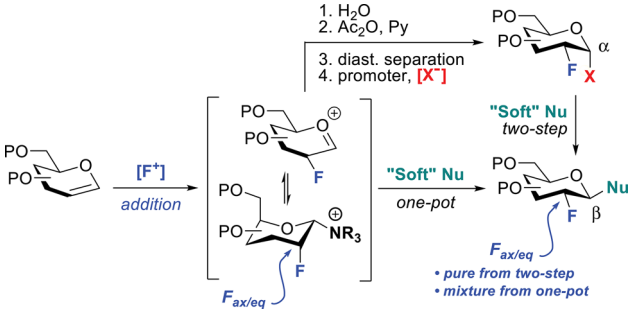
Supporting Information

ABSTRACT: 2-Deoxy-2-fluoroglycosyl iodides are privileged glycosyl donors for the stereoselective preparation of 1-Nu-β-fluorosugars, which are useful reagents for chemical site-selective protein glycosylation. Ready access to such β-fluorosugars enables the mild and efficient construction of well-defined fluoroglycoproteins.



F-glycopeptides[1−9] and the more recently disclosed F-glycoproteins[10−19] are promising candidates for the preparation of synthetic carbohydrate vaccines[20] and hold great potential as a new generation of [^{18}F]-(glyco)radiopharmaceuticals[21,22] and tracers for the noninvasive imaging techniques positron emission tomography (^{18}F-PET)[23,24] and magnetic resonance/magnetic resonance imaging (^{19}F-MR/MRI).[25] In addition to the development of more efficient methods for protein/peptide modification,[26] it is also pivotal to access pure F-sugar reagents and building blocks for such transformations. These glycosyl units typically include in their structure reactive handles such as azides (for copper(I)-catalyzed azide−alkyne cycloaddition−CuAAC and Staudinger ligations)[17] or chalcogens in the form of sulfhydryl groups or diselenide moieties (for exchange reactions with Cys or additions to dehydroalanine),[14,15] which upon reaction generate a new carbohydrate−protein conjugate. Retrosynthetic analysis revealed that 2-F-glycosyl halides are suitable glycosyl donors for the preparation of such reagents via biphasic glycosylations/phase-transfer catalyzed reactions (PTC). These moieties enable exquisite control of β-anomeric selectivity (via S_N2-like mechanism) during the introduction of the reactive handle, which is typically a "soft" nucleophilic moiety.[27] There are two general strategies (one-pot from glycals vs two-step from 2-F-pyranoses) for the preparation of 1-β-"soft Nu"-2-deoxy-2-fluoroglycosides using electrophilic F^+ reagents (Scheme 1).[28] While the one-pot strategy works well for certain configurations (e.g., Gal), the two-step approach is more general and thus preferred for the preparation of pure F-sugar reagents in a more efficient and homogeneous manner (giving a better overall yield and controlled $F_{ax/eq}$ diastereoselectivity).

Scheme 1. General Strategies for the Preparation of 1-β-"Soft Nu"-2-deoxy-2-fluoroglycosides Using F^+



Among glycosyl halides, iodides[29] have been recently utilized in several glycosylation strategies and provide a robust platform for PTC reactions, perhaps leading to higher yields compared to chlorides or bromides due to their superior leaving group properties under S_N2-like reaction conditions. There are very few examples of 2-F- and 2,2-diF-glycosyl iodides. This rare class of glycosyl donors include some isolated examples of 2-F- and 2,2-diF-pyranosides[30−32] and our 2-F-galactopyranosyl iodide prepared via a "one-pot" strategy, as revealed by VT-NMR experiments (see Supporting Information),[33] and more recently 2,2-diF-furanosides.[34] Despite their great potential, low preparative yields in the pyranose series and inseparable α/β mixtures in the case of furanoses has so far hampered their wide utilization as β-selective glycosyl donors in PTC protocols.

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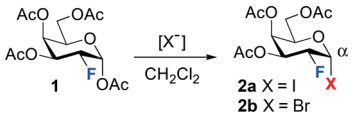
In this work, we envisioned a "two-step" strategy for the preparation of 2-F-glycosyl iodides from ready available 2-F-glycosyl-1-*O*-Ac derivatives that might overcome such limitations enabling a more efficient preparation of this class of highly reactive glycosyl donors. We then sought to explore their reactivity for the preparation of fluorosugar reagents that would serve as useful moieties to achieve site-selective chemical glycosylation of proteins. We first evaluated the reaction conditions using 2-F-α/β-galactopyranose **1** (Table 1). Initial

Table 1. Optimization of Reaction Conditions for the Halogenation of 1[a]

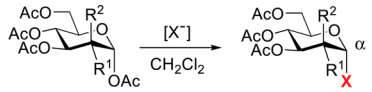


entry	conditions (equiv)	product	yield (%)
1	TMSI (1), rt, 8 h	**2a**	57[b]
2	TMSI (1.1), MgO (2), reflux, 12 h	**2a**	78[b]
3	TMSI (2.2), MgO (4), rt, 24 h	**2a**	85
4	AcSH (1.7), I_2 (0.8), MgO (2), reflux, 30 h	**2a**	24[b]
5	AcSH (4.4), I_2 (2), reflux, 12 h	**2a**	34[b]
6	AcSH (2.2), I_2 (1), reflux, 12 h	**2a**	54[b]
7	HMDS (0.6), I_2 (0.6), rt, 24 h	**2a**	mixture
8	33% HBr in AcOH, rt, 8 h	**2b**	95

[a]General conditions: 2-F-α/β-galactopyranose **1** (2:1 α/β) (1 equiv) in dry CH_2Cl_2 (0.1 M) unless otherwise indicated. [b]Isolated yield after two consecutive reaction cycles. TMS = trimethylsilyl, HMDS = hexamethyldisilazane.

experiments revealed TMSI[35] to be an effective promoter for the preparation of glycosyl iodide **2a**, although the reaction only proceeded in 57% yield after two consecutive reaction cycles (entry 1). We found that addition of MgO[36] that acts as a scavenger for the *in situ* generated TMSOAc avoids the reversible formation of starting **1**, while increasing the yields up to 78% (entry 2). Good yields (up to 85% in a single reaction cycle) were finally obtained by increasing the amount of both TMSI (2.2 equiv) and MgO (4 equiv) as well as reducing the reaction temperature (from reflux to rt) in order to avoid detrimental side reactions (entry 3). Other promoters were also evaluated (entries 4−7). While the use of HMDS/I_2 proved ineffective and complex mixtures of products were observed (entry 7),[37] the *in situ* formation of highly reactive HI by refluxing AcSH and I_2 allowed the formation of the desired iodide **2a**, albeit in lower yields compared to TMSI (up to 54%) (entries 4−6).[38] Interestingly, the use of MgO was found to be costly (24%) when using AcSH and I_2 probably due to its incompatibility with AcSH and/or HI (entry 4). 2-F-galactosyl bromide **2b** was also prepared following a reported procedure in 95% yield (Table 1, entry 8).[39] With the optimized conditions in hand we set out to evaluate the scope of the iodination with other configurations (Table 2). Conditions A (with TMSI) were utilized with 2-F-α/β-pyranoses of *Glc* **3** and *Man* **4** configuration affording desired glycosyl iodides **5a** and **6a** in moderate yields (entries 1 and 4). To our delight, conditions B (with AcSH and I_2) improved the yield to 82% for **5a** and 75% for **6a** (entries 2 and 5). 2-F-glycosyl bromides **5b** and **6b** were also prepared in excellent yields (up to 98%) using the standard conditions (entries 3 and 6). Practical yields of the corresponding α-glycosyl iodides as sole anomers (e.g., $^1J_{C1-H1}$ = 187.6 Hz, $^3J_{1,2}$ = 4.7 Hz, and $^3J_{3,4}$ = 10.2 Hz in **5a**, $^4C_1$

Table 2. Scope of Halogenation[a]



3 R^1 = F, R^2 = H (*Glc*) **4** R^1 = H, R^2 = F (*Man*) **5a** R^1 = F, R^2 = H, X = I; **5b** X = Br **6a** R^1 = H, R^2 = F, X = I; **6b** X = Br

entry	2-F-pyranose	conditions	product	yield (%)
1	**3**	A	**5a**	38[b]
2	**3**	B	**5a**	82[b]
3	**3**	C	**5b**	98
4	**4**	A	**6a**	50[b]
5	**4**	B	**6a**	75[b]
6	**4**	C	**6b**	94

[a]General conditions: 2-F-α/β-pyranose (1:0−1:3 α/β) (1 equiv) in dry CH_2Cl_2 (0.1 M) unless otherwise indicated. [b]Isolated yield after two consecutive reaction cycles. Conditions A: TMSI (2.2 equiv), MgO (4 equiv), rt, 30 h. Conditions B: AcSH (2.2), I_2 (1), reflux, 5 h. Conditions C: 33% HBr in AcOH, rt, 3−8 h. TMS = trimethylsilyl.

conformation)[40] were obtained regardless of the pyranoside configuration (*Gal*, *Glc*, and *Man*).

2-F-glycosyl iodides proved stable and were purified by SiO_2 flash column chromatography with no decomposition observed. They are also UV visible (at 254 nm) providing an easy experimental follow-up. Similar to other protocols that use strong acid activators (e.g., HBr), base sensitive acetyl groups are the more appropriate protecting groups for the overall synthetic route (Selectfluor−halogenation−PTC−deprotection). Acetyl protecting groups are easily introduced, prove stable under the sequence of transformations, and are easily removed under Zemplén conditions to afford the final unprotected and ready-to-conjugate fluorosugar reagents. Other acid sensitive groups such as *tert*-butyldiphenylsilyl (TBDPSi) were not tolerated under the optimized acid−based reaction conditions, and extensive deprotection was observed.

Next, the synthesis of 1-β-"soft Nu"-2-deoxy-2-fluoroglycosides was attempted using 2-F-glycosyl halides **2a,b** and **5**−**6a,b** (Scheme 2). A wide range of *N*-, *S*-, and *Se*-nucleophiles led to β-fluorosugar reagents **7**−**10**, useful for chemical protein glycosylation strategies,[14,15,17] even including those with the challenging 1-β-*cis*-*Man*[41] disposition **9a**−**c**. Excellent yields (up to 98%) and pure β-selectivity (e.g., $^1J_{C1-H1}$ = 162.7 Hz, $^3J_{1,2}$ = 10.6 Hz, and $^3J_{3,4}$ = 9.5 Hz in **8c**, $^4C_1$ conformation)[40] were always obtained. Iodides proved slightly better in terms of yield (ca. 10−15%) over their bromide counterparts using a better Nu in a "match" scenario of reactivity. We believe the low reactivity of mannosyl-1-halides is probably due to unfavorable dipolar interactions between the donor and the incoming Nu and/or conformational issues that reduce the reaction rate. This made competitive otherwise negligible side reactions that reduce the overall yield.[42] A weaker Nu such as sodium benzenethiosulfonate (NaPTS)[43] necessitated the use of more reactive iodides and afforded moderate-to-good yields (88% for I vs 45% for Br in **10a**). Our data indicate that 2-F-glycosyl iodides are needed when using weaker nucleophiles. The reported 2-F-glycosyl iodides may also find broader utility for example in the preparation of advanced fluorinated building blocks such as *O*-linked natural product mimetics[44] **11a** and covalent inhibitors[45,46] of carbohydrate processing enzymes **11b** and **12** where the use of "soft" nucleophilic acceptors are required. Finally, to illustrate the application of such reagents and further complement our previous reports on the

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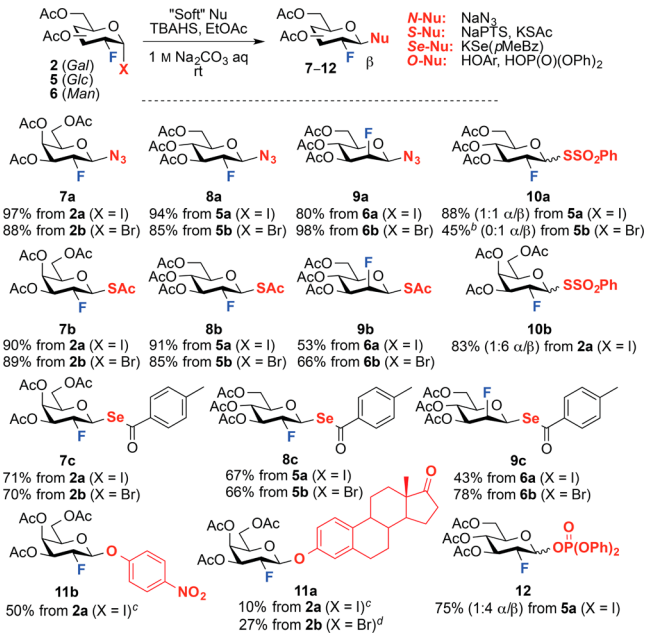
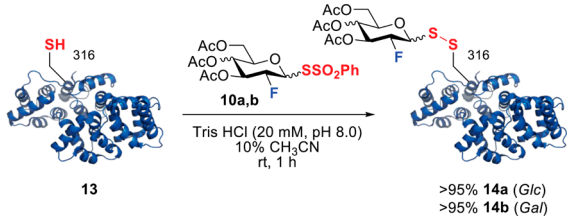
Scheme 2. Phase-Transfer Catalyzed Reactions (PTC) for the Preparation of 7–12[a]



[a]General conditions: 2-F-α-pyranosyl halide (1 equiv), Nu (2 equiv), TBAHS (2 equiv) in 5:1 EtOAc–1 M Na_2CO_3 aq (0.04 M), rt, from 1 h up to 7 days unless otherwise indicated. [b]Determined by ^{19}F NMR of the crude reaction mixture. [c]2-F-α-galactosyl bromide **2b** (1 equiv), Nu (2 equiv), TBAB (2 equiv) in 3:2 CH_2Cl_2–5% NaOH aq (0.02 M), rt, 14–22 h. [d]2-F-α-galactosyl bromide **2b** (2 equiv), estrone (1 equiv), 60% NaH in mineral oil (1.5 equiv) in dry 1,4-dioxane (0.08 M), rt, 24 h. Nu = nucleophile. TBAHS = tetrabutylammonium hydrogen sulfate. TBAB = tetrabutylammonium bromide.

preparation of a variety of well-defined triazole,[17] thioether,[15,18] and *SeS*-linked[14,18] fluorinated glycoproteins including their [^{18}F]-counterparts, we examined the reaction between Cys-specific Ac_3GlcF[9] and Ac_3GalF-1-PTS reagents **10a,b** with Annexin V **13**. This protein displays a unique naturally occurring surface exposed Cys and is widely utilized as a marker of apoptosis (Scheme 3).[47] Indeed, reaction of **10a** or

Scheme 3. Selective Chemical Protein Modification of Annexin V 13[a]



[a]General conditions: Annexin V **13** (1 mg mL^{-1}) (1 equiv), 2-F-sugar reagent **10a,b** (250 equiv) in 20 mM Tris HCl buffer (pH 8.0) and 10% CH_3CN, rt, 1 h.

10b with Annexin V **13** in 20 mM Tris HCl buffer pH 8 and 10% CH_3CN, which was used to ensure reagent solubility, gave the expected homogeneous disulfide-linked F-glycoproteins **14a,b** in >95% conversion after 1 h at rt. This is demonstrative of the utility of the reagents that can now be easily prepared from the 2-F-glycosyl iodide reagents we have reported in this work. Moreover, this methodology is amenable to utilization in ^{18}F-PET imaging using the corresponding [^{18}F]-glyco-1-PTS reagents.[9]

In summary, we have disclosed a general strategy for accessing a wide range of 1-Nu-2-fluorosugars that are useful reagents for chemical-site-selective protein glycosylation. The glycosyl iodide intermediates prepared here possess a seemly balance between stability and reactivity that facilitates their preparation, purification, and storage. Importantly, these intermediates also ensure product homogeneity by their exquisite β-control during stereoselective glycosylation with a "soft" Nu (via S_N2-like reactions). The reported F-sugar reagents will find broad utility for building not only homogeneous ^{19}F- but also [^{18}F]-glycoprobes that are valuable tools in the fields of chemical biology and biomedical imaging.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, VT-NMR experiments, characterization data, and copies of ^1H, ^{13}C, and ^{19}F NMR spectra for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01259.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Johannes, M.; Reindl, M.; Gerlitzki, B.; Schmitt, E.; Hoffmann-Röder, A. *Beilstein J. Org. Chem.* **2015**, *11*, 155–161.
(2) Lamandé-Langle, S.; Collet, C.; Hensienne, R.; Vala, C.; Chrétien, F.; Chapleur, Y.; Mohamadi, A.; Lacolley, P.; Regnault, V. *Bioorg. Med. Chem.* **2014**, *22*, 6672–6683.
(3) Dall'Angelo, S.; Zhang, Q.; Fleming, I. N.; Piras, M.; Schweiger, L. F.; O'Hagan, D.; Zanda, M. *Org. Biomol. Chem.* **2013**, *11*, 4551–4558.
(4) Lang, C.; Maschauer, S.; Hübner, H.; Gmeiner, P.; Prante, O. *J. Med. Chem.* **2013**, *56*, 9361–9365.
(5) Li, X.-G.; Dall'Angelo, S.; Schweiger, L. F.; Zanda, M.; O'Hagan, D. *Chem. Commun.* **2012**, *48*, 5247–5249.
(6) Maschauer, S.; Einsiedel, J.; Haubner, R.; Hocke, C.; Ocker, M.; Hübner, H.; Kuwert, T.; Gmeiner, P.; Prante, O. *Angew. Chem., Int. Ed.* **2010**, *49*, 976–979.
(7) Hoffmann-Röder, A.; Kaiser, A.; Wagner, S.; Gaidzik, N.; Kowalczyk, D.; Westerlind, U.; Gerlitzki, B.; Schmitt, E.; Kunz, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 8498–8503.
(8) Wuest, F.; Hultsch, C.; Berndt, M.; Bergmann, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5426–5428.
(9) Prante, O.; Einsiedel, J.; Haubner, R.; Gmeiner, P.; Wester, H.-J.; Kuwert, T.; Maschauer, S. *Bioconjugate Chem.* **2007**, *18*, 254–262.

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Org. Lett. 2015, 17, 2836–2839

(10) Huo, C.-X.; Zheng, X.-J.; Xiao, A.; Liu, C.-C.; Sun, S.; Lv, Z.; Ye, X.-S. *Org. Biomol. Chem.* **2015**, *13*, 3677−3690.
(11) Lee, H.-Y.; Chen, C.-Y.; Tsai, T.-I.; Li, S.-T.; Lin, K.-H.; Cheng, Y.-Y.; Ren, C.-T.; Cheng, T.-J. R.; Wu, C.-Y.; Wong, C.-H. *J. Am. Chem. Soc.* **2014**, *136*, 16844−16853.
(12) Orwenyo, J.; Huang, W.; Wang, L. X. *Bioorg. Med. Chem.* **2013**, *21*, 4768−4777.
(13) Fröhlich, R. F. G.; Schrank, E.; Zangger, K. *Carbohydr. Res.* **2012**, *361*, 100−104.
(14) Boutureira, O.; Bernardes, G. J. L.; Fernández-González, M.; Anthony, D. C.; Davis, B. G. *Angew. Chem., Int. Ed.* **2012**, *51*, 1432−1436.
(15) Boutureira, O.; Bernardes, G. J. L.; D'Hooge, F.; Davis, B. G. *Chem. Commun.* **2011**, *47*, 10010−10012.
(16) Yang, F.; Zheng, X.-J.; Huo, C.-X.; Wang, Y.; Zhang, Y.; Ye, X.-S. *ACS Chem. Biol.* **2011**, *6*, 252−259.
(17) Boutureira, O.; D'Hooge, F.; Fernández-González, M.; Bernardes, G. J. L.; Sánchez-Navarro, M.; Koeppe, J. R.; Davis, B. G. *Chem. Commun.* **2010**, *46*, 8142−8144.
(18) Fernández-González, M.; Boutureira, O.; Bernardes, G. J. L.; Chalker, J. M.; Young, M. A.; Errey, J. C.; Davis, B. G. *Chem. Sci.* **2010**, *1*, 709−715.
(19) Wuest, F.; Berndt, M.; Bergmann, R.; van den Hoff, J.; Pietzsch, J. *Bioconjugate Chem.* **2008**, *19*, 1202−1210.
(20) Adamo, R.; Nilo, A.; Castagner, B.; Boutureira, O.; Berti, F.; Bernardes, G. J. L. *Chem. Sci.* **2013**, *4*, 2995−3008.
(21) Maschauer, S.; Prante, O. *Biomed Res. Int.* **2014**, *2014*, Article ID 214748.
(22) Ribeiro Morais, G.; Falconer, R. A.; Santos, I. *Eur. J. Org. Chem.* **2013**, 1401−1414.
(23) Jacobson, O.; Kiesewetter, D. O.; Chen, X. *Bioconjugate Chem.* **2015**, *26*, 1−18.
(24) Ametamey, S. M.; Honer, M.; Schubiger, P. A. *Chem. Rev.* **2008**, *108*, 1501−1516.
(25) Tirotta, I.; Dichiarante, V.; Pigliacelli, C.; Cavallo, G.; Terraneo, G.; Bombelli, F. B.; Metrangolo, P.; Resnati, G. *Chem. Rev.* **2015**, *115*, 1106−1129.
(26) Boutureira, O.; Bernardes, G. J. L. *Chem. Rev.* **2015**, *115*, 2174−2195.
(27) Carrière, D.; Meunier, S. J.; Tropper, F. D.; Cao, S.; Roy, R. *J. Mol. Catal. A: Chem.* **2000**, *154*, 9−22.
(28) Dax, K.; Albert, M.; Ortner, J.; Paul, B. J. *Carbohydr. Res.* **2000**, *327*, 47−86.
(29) Meloncelli, P. J.; Martin, A. D.; Lowary, T. L. *Carbohydr. Res.* **2009**, *344*, 1110−1122.
(30) Nakai, K.; Takagi, Y.; Tsuchiya, T. *Carbohydr. Res.* **1999**, *316*, 47−57.
(31) Premchandran, R. H.; Ogletree, M. L.; Fried, J. *J. Org. Chem.* **1993**, *58*, 5724−5731.
(32) Tsuchiya, T.; Takahashi, Y.; Endo, M.; Umezawa, S.; Umezawa, H. *J. Carbohydr. Chem.* **1985**, *4*, 587−611.
(33) Boutureira, O.; Díaz, Y.; Matheu, M. I.; Castillón, S. Synthesis and reactivity of 2-deoxy-2-fluoroglycosyl iodides. Part of this work has been presented as a poster at the 23rd International Carbohydrate Symposium, Whistler, Canada, July 23−28, 2006; TUE-PS.6.
(34) Chu, C.-Y.; Lee, W.-D.; Li, W.; Hwang, C. K. PCT Int. Appl. WO 2009061894A1 20090514, 2009.
(35) Thiem, J.; Meyer, B. *Chem. Ber.* **1980**, *113*, 3075−3085.
(36) Dabideen, D. R.; Gervay-Hague, J. *Org. Lett.* **2004**, *6*, 973−975.
(37) Mukhopadhyay, B.; Kartha, K. P. R.; Russell, D. A.; Field, R. A. *J. Org. Chem.* **2004**, *69*, 7758−7760.
(38) Chervin, S. M.; Abada, P.; Koreeda, M. *Org. Lett.* **2000**, *2*, 369−372.
(39) Kováč, P.; Yeh, H. J. C.; Glaudemans, C. P. J. *Carbohydr. Res.* **1987**, *169*, 23−34.
(40) Michalik, M.; Hein, M.; Frank, M. *Carbohydr. Res.* **2000**, *327*, 185−218.
(41) Abronina, P. I.; Kachala, V. V.; Kononov, L. O. *Carbohydr. Res.* **2009**, *344*, 240−244.
(42) Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A. *Org. Lett.* **2014**, *16*, 4838−4841.
(43) Gamblin, D. P.; Garnier, P.; Ward, S. J.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Org. Biomol. Chem.* **2003**, *1*, 3642−3644.
(44) Černý, I.; Novotný, P.; Drašar, P.; Havel, M.; Pacák, J. *Collect. Czech. Chem. Commun.* **1989**, *54*, 2775−2783.
(45) Rempel, B. P.; Withers, S. G. *Org. Biomol. Chem.* **2014**, *12*, 2592−2595.
(46) Rempel, B. P.; Tropak, M. B.; Mahuran, D. J.; Withers, S. G. *Angew. Chem., Int. Ed.* **2011**, *50*, 10381−10383.
(47) Logue, S. E.; Elgendy, M.; Martin, S. J. *Nat. Protoc.* **2009**, *4*, 1383−1395.

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