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-glycopeptides1−9 and the more recently disclosed F-glycoproteins10−19 are promising candidates for the preparation of synthetic carbohydrate vaccines20 and hold great potential as a new generation of [18F]-(glyco)radiopharmaceuticals21,22 and tracers for the noninvasive imaging techniques positron emission tomography (18F-PET)23,24 and magnetic resonance/magnetic resonance imaging (19F-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 SN2-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 Fα/β diastereoselectivity).

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

Among glycosyl halides, iodides29 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 SN2-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-pyranosides30−32 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.
In this work, we envisioned a “two-step” strategy for the preparation of 2-F-glycosyl iodides from readily 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 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 formation of highly reactive HI by reactions that reduce the overall yield.\(^{42}\) A weaker Nu such as sodium benzenethiosulfonate (NaPTS)\(^{43}\) necessitated the use of 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-butylidihexylsilyl (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 5a–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 \(9\)–c. Excellent yields (up to 98\%) and pure β-selectivity (e.g., \(J_{\text{C1-\text{H1}}} = 162.7\) Hz, \(J_{\text{J2-\text{H2}}} = 10.6\) Hz, and \(J_{\text{J3-\text{H3}}} = 9.5\) Hz in 8c, \(\alpha\)C1 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)\(^{44}\) 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 ω-linked natural product mimetics\(^{44}\) 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 glycosylation of proteins. We \(\alpha\)-glycosyl iodides were as ole anomers (e.g., \(J_{\text{C1-\text{H1}}} = 187.6\) Hz, \(J_{\text{J1-\text{H1}}} = 4.7\) Hz, and \(J_{\text{J3-\text{H3}}} = 10.2\) Hz in 5a, \(\alpha\)C1 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).

### Table 1. Optimization of Reaction Conditions for the Halogenation of 1

<table>
<thead>
<tr>
<th>entry</th>
<th>conditions (equiv)</th>
<th>product</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TMSI (1), rt, 8 h</td>
<td>2a</td>
<td>57(^{b})</td>
</tr>
<tr>
<td>2</td>
<td>TMSI (1.1), MgO (2), reflux, 12 h</td>
<td>2a</td>
<td>78(^{b})</td>
</tr>
<tr>
<td>3</td>
<td>TMSI (2.2), MgO (4), rt, 24 h</td>
<td>2a</td>
<td>85</td>
</tr>
<tr>
<td>4</td>
<td>AcSH (1.7), I(_2) (0.8), MgO (2), reflux, 30 h</td>
<td>2a</td>
<td>24(^{b})</td>
</tr>
<tr>
<td>5</td>
<td>AcSH (4.4), I(_2) (2), reflux, 12 h</td>
<td>2a</td>
<td>34(^{b})</td>
</tr>
<tr>
<td>6</td>
<td>AcSH (2.2), I(_2) (1), reflux, 12 h</td>
<td>2a</td>
<td>54(^{b})</td>
</tr>
<tr>
<td>7</td>
<td>HMDS (0.6), I(_2) (0.6), rt, 24 h</td>
<td>mixture</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>33% HBr in AcOH, rt, 8 h</td>
<td>2b</td>
<td>95</td>
</tr>
</tbody>
</table>

\(^{a}\)General conditions: 2-F-α/β-galactopyranose 1 (2:1 α/β (1 equiv) in dry CH\(_2\)Cl\(_2\) (0.1 M) unless otherwise indicated.\(^{\text{b}}\)Isolated yield after two consecutive reaction cycles. TMS = trimethylsilyl, HMDS = hexamethyldisilazane.

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Scheme 2. Phase-Transfer Catalyzed Reactions (PTC) for the Preparation of 7−12

![Diagram of Scheme 2](image)

General conditions: 2-F-α-pyroanyl halide (1 equiv), Nu (2 equiv), TBAHS (2 equiv) in 5:1 EtOAc−1 M Na2CO3 aq (0.04 M), rt, from 1 h up to 7 days unless otherwise indicated. Determined by 19F NMR of the crude reaction mixture. 2-F-α-galactosyl bromide 2b (1 equiv), Nu (2 equiv), TRAB (2 equiv) in 3:2 CH2Cl2−5% NaOH aq (0.02 M), rt, 14−22 h. 2-F-α-galactosyl bromide 2b (2 equiv), estrone (1 equiv), 2-F-sugar (250 equiv) in 20 mM Tris HCl buffer (pH 8.0), rt, 1 h.

preparation of a variety of well-defined triazole, thioether, and SeS-linked fluorinated glycoproteins including their [18F]-counterparts, we examined the reaction between Cys-specific Ac2GlcP and Ac2GaF-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). Indeed, reaction of 10a or 10b with Annexin V 13 in 20 mM Tris HCl buffer pH 8 and 10% CH3CN, 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 18F-PET imaging using the corresponding [18F]-glyco-1-PTS reagents.

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 seemingly 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 SN2-like reactions). The reported F-sugar reagents will find broad utility for building not only homogeneous 18F- but also [18F]-glycopolypeptides 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, 13C, and 18F 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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