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One-Pot Strategies for the Synthesis of the Tetrasaccharide Linkage Region of Proteoglycans

Teng-Yi Huang,[†,‡] Medel Manuel L. Zulueta,[†] and Shang-Cheng Hung[*,†,§]

Genomics Research Center, Academia Sinica, 128 Sec.2 Academia Road, Taipei 115, Taiwan, Department of Chemistry, National Tsing Hua University, 101 Sec. 2 Kuang-Fu Road, Hsinchu 300, Taiwan, and Department of Applied Chemistry, National Chiao Tung University, 1001 Ta-Hsueh Road, Hsinchu 300, Taiwan

schung@gate.sinica.edu.tw

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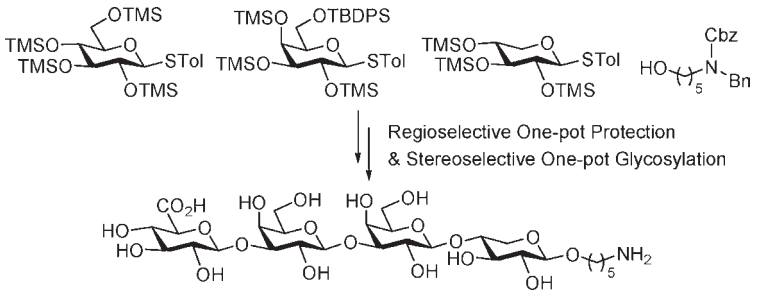
ABSTRACT



A linker-attached tetrasaccharide corresponding to the linkage region of proteoglycans was synthesized via one-pot procedures from the silylated monosaccharide derivatives. Regioselective one-pot protection protocols were applied in generating the requisite monosaccharide building blocks whereas stereoselective one-pot glycosylation approaches were utilized to assemble the tetrasaccharide skeleton.

Proteoglycans are biologically important macromolecules widespread on the cell surface and in the extracellular matrix.[1] They interact with other biomolecules through the distinct profile of the glycosaminoglycan (GAG) chains decorating the protein core.[2] GAG biosynthesis initiates from the β-attachment of D-xylose (Xyl) to a serine residue followed by consecutive additions of two D-galactose (Gal) and one D-glucuronic acid (GlcA) unit in the respective $\beta1\rightarrow4$, $\beta1\rightarrow3$, and $\beta1\rightarrow3$ manner generating the tetrasaccharide linkage region (Figure 1).[3] Further chain elongation sorts GAGs into two major categories. Heparin and heparan sulfate form by the alternate additions of *N*-acetyl D-glucosamine and GlcA whereas chondroitin sulfate and dermatan sulfate incorporate *N*-acetyl D-galactosamine

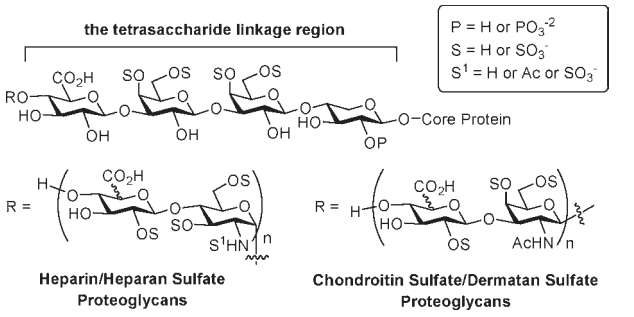


Figure 1. Structures of proteoglycans.

[†] Genomics Research Center, Academia Sinica.
[‡] Department of Chemistry, National Tsing Hua University.
[§] Department of Applied Chemistry, National Chiao Tung University.

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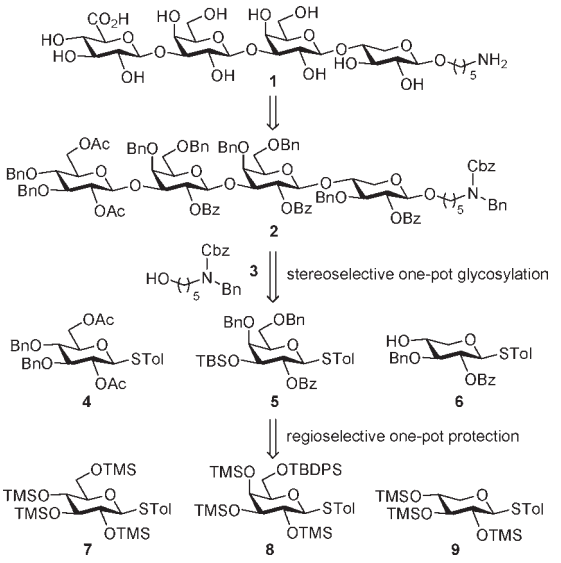
and GlcA.[4] Intermittent *N*-deacetylation, uronic acid 5-*C*-epimerization, and multiple *N*- and *O*-sulfonations deliver the mature structure responsible for biological activity.

The formation of different GAGs with high fidelity from a common protein-linked tetrasaccharide precursor has intrigued many investigators. Occasional modifications, such as 2-*O*-phosphorylation of Xyl and 4- and 6-*O*-sulfonations of Gal residues, were suggested to influence the divergent biosynthetic routes.[5] Understanding the nature of these modifications and their effects on the specificity of biosynthetic enzymes require compounds that mimic the natural substrates. Synthetic methodologies that target the proteoglycan linkage region backbone have been reported.[6] Notable concerns therein include the tedious generation of appropriately protected monosaccharide building blocks, particularly that of Xyl,[7] and the stereocontrol in the Galβ1→3Gal glycosidic bond formation that often give low selectivity and yield.[8] We recently demonstrated one-pot strategies for regioselective protection of monosaccharides and stereoselective glycosylation in order to simplify synthetic procedures and reduce time- and resource-consuming workup and purification steps.[9] Drawing from these methodologies, we present herein an approach to the chemical synthesis of the linkage region tetrasaccharide **1** fitted with an amine terminated linker as an aid to deciphering the processes associated with the modification of the linkage region together with its roles in chain elongation.

Our retrosynthetic plan is depicted in Scheme 1. By typical transformations, compound **1** is accessible following the assembly of the fully protected tetrasaccharide **2** using stereoselective one-pot glycosylations of the monosaccharide building blocks **4**−**6** and the linker derivative **3**. The orthogonal TBS protection of the thiogalactoside **5** would facilitate chain elongation and is also expected to enhance the donor reactivity during the coupling processes.

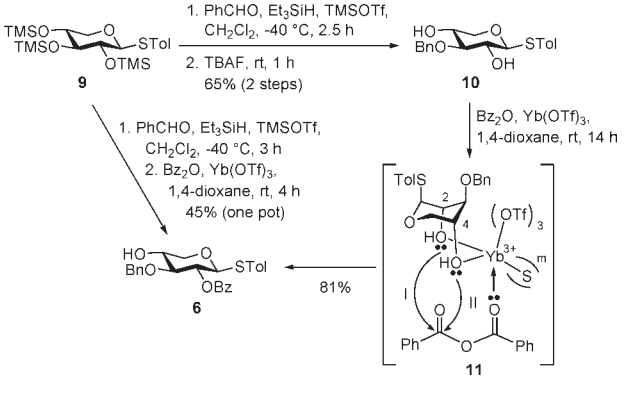
Scheme 1. Retrosynthesis of Compound **1**



The β-selectivity in forming all glycosidic bonds would rely on neighboring group assistance by the acyl groups at 2-O of **4**−**6**; solvent effects could be exploited in pertinent cases. Compounds **4**−**6** would be prepared through regioselective one-pot protection strategies starting from the silylated thioglycosides **7**−**9**, respectively.

The nearly similar reactivities of the 2-C, 3-C, and 4-C hydroxyls of D-xylopyranosides render their full differentiation a challenging task. Delightfully, the TMSOTf-catalyzed Et_3SiH-reductive benzylation of the 2,3,4-tri-*O*-TMS ether **9** primarily gave, after TBAF treatment, the 3-*O*-benzylated **10**. At −40 °C with 1.1 equiv of benzaldehyde, **10** was obtained in 65% yield (Scheme 2). Its 2- and 4-OBn isomers were also isolated at 10% and 6% yields, respectively. We next focused on regioselective benzoyl group installation at the 2-O position. Treatment of **10** with BzCl in pyridine or Bz_2O in the presence of TMSOTf formed the 4-*O*-benzoylated isomer as the major product in 40% and 30% yields respectively. Recently, we found that $Yb(OTf)_3$-catalyzed acylation was effective in

Scheme 2. Synthesis of the Thioxyloside **6**



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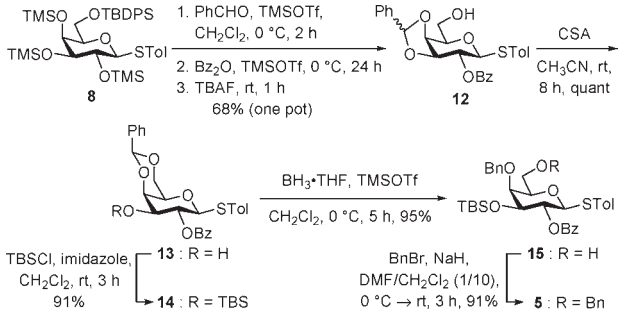
the desymmetrization of *myo*-inositol 1,3,5-orthoformate.[10] Benzoylation with 5 mol % $Yb(OTf)_3$ and 1.05 equiv of Bz_2O provided good regioselectivity, and the 4-alcohol **6** was isolated in 81% yield. The metal ion perhaps coordinates with both 2-O and 4-O atoms forcing the $^1C_4$ conformation in the intermediate **11**, enhancing regioselective Bz group introduction at the more nucleophilic 2-O (path I) rather than the 4-O position (path II). When benzylation (PhCHO, Et_3SiH, TMSOTf) and benzoylation [Bz_2O, $Yb(OTf)_3$] were carried out in one pot from **9**, product **6** was obtained in 45% isolated yield. To our knowledge, this is, by far, the most straightforward preparation of xylosyl derivatives of identical protecting group pattern.

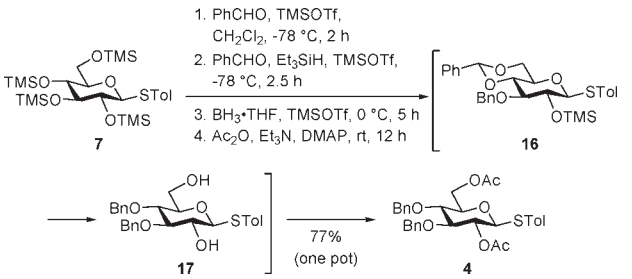
Scheme 3. Synthesis of the Thiogalactoside **5**



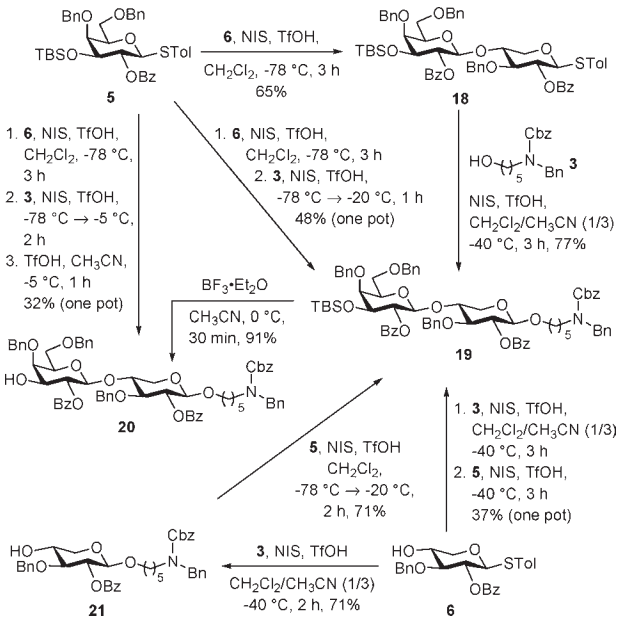
Unlike the similar case for D-glucopyranosides where 2-*O*-acylations predominate,[11] one-pot benzylidenation and monobenzoylation of *p*-methylphenyl 2,3,4,6-tetra-*O*-TMS-1-thio-β-D-galactopyranoside gave us the unwanted 3-*O*-benzoyl derivative as the major isomer (62%). Alternatively, we performed the protecting group installation through the TBDPS functionalized **8** (Scheme 3). With catalytic TMSOTf, **8** was subjected to 3,4-*O*-benzylidenation, 2-*O*-benzoylation, and 6-*O*-desilylation in one pot to afford the 6-alcohols **12** (68%, *exo*/*endo* = 1/1.1). Treatment of **12** with 10-camphorsulfonic acid (CSA) led to benzylidene migration quantitatively providing the 3-alcohol **13** which, in turn, was masked with the TBS group to form the fully protected **14** in 91% yield. Although **14** carries applicable protecting groups for chain assembly, our preliminary trials for the β1→3 bond formation between the Gal residues gave low yields, which might be caused by the rigid and bulky 4,6-*O*-benzylidene group. Accordingly, regioselective acetal ring-opening using BH_3•THF and TMSOTf was performed furnishing the 6-alcohol **15** (95%), which underwent Williamson etherification to generate the galactosyl donor **5** in 91% yield. It was noted, in this special example, that the 2-*O*-benzoyl group tolerated the basic conditions.

Scheme 4 illustrates the regioselective one-pot synthesis of the D-*gluco* β-thiopyranoside **4** from the 2,3,4,6-tetra-*O*-TMS ether **7** in 77% yield. Through TMSOTf catalysis, **7** underwent 4,6-*O*-benzylidenation followed by reductive 3-*O*-benzylation to furnish the intermediate **16**. In the same flask, the 6-*O*-ring-opening of the benzylidene acetal still catalyzed by TMSOTf, employing BH_3•THF as the reductant, generated the 2,6-diol intermediate **17**. Then, the reaction mixture was treated with Et_3N to pave the way for the 2,6-di-*O*-acetylation in the presence of DMAP.

Scheme 4. Synthesis of the Thioglucoside **4**



Scheme 5. Synthesis of the Disaccharide Acceptor **20**



With the key building blocks **4**−**6** in hand, the construction of the target sugar backbone was initiated (Scheme 5). Following *N*-iodosuccinimide (NIS) and TfOH treatment, selective activation of the thiogalactoside **5** in the presence

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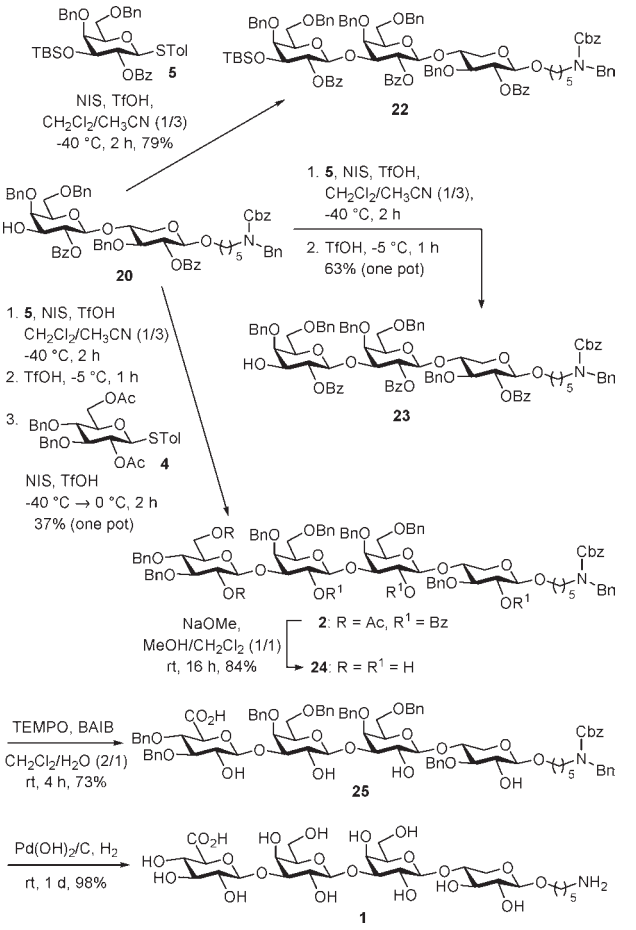
of the thioxyloside **6** was achieved at −78 °C giving the β-disaccharide **18** (65%, $J_{1',2'}$ = 8.1 Hz). Further condensation with the linker derivative **3**[12] formed the adduct **19** ($J_{1,2}$ = 7.1 Hz) in 77% yield. When this sequence was done in one pot, **19** was acquired in an isolated yield of 48%. For the necessary TBS group cleavage, acidic hydrolysis[13] was utilized to prevent the base-mediated Bz migration. Consequently, the 3-alcohol **20** (91%) was efficiently acquired after treatment of **19** with BF_3•Et_2O. Extension of the one-pot procedure to the TBS deprotection via TfOH treatment gave the required compound **20** in 32% yield.

We next turned to the alternative reducing end-to-nonreducing end route. Taking advantage of the higher reactivity of the primary alcohol in **3**, its glycosylation at −40 °C by the thioxyloside **6** delivered the β-linked **21** ($J_{1,2}$ = 7.0 Hz) in 71% yield. Coupling of **21** with the thiogalactoside **5** led to compound **19** (71%). Glycosylation of **3** with **6** followed by coupling with the donor **5** in one pot at the same temperature provided **19** in 37% yield. Unfortunately, inclusion of the TfOH-mediated cleavage of the TBS group in the one-pot method resulted in products which are difficult to purify.

The preparation of the target tetrasaccharide is depicted in Scheme 6. The stereoselectivity of the NIS/TfOH-activated coupling of the thiogalactoside **5** and the disaccharide acceptor **20** was improved from a β/α ratio of 3/1 with CH_2Cl_2 as solvent to 14/1 when the CH_2Cl_2/CH_3CN (1/3) solvent mixture[14] was utilized; the trisaccharide **22** ($J_{1'',2''}$ = 7.7 Hz) was obtained in 79% yield. When this glycosylation, followed by acidic desilylation, was carried out in one pot, the 3″-alcohol **23** was isolated in 63% yield. The thioglucoside **4** activation with NIS and TfOH was further included in the one-pot process which led to the linkage region tetrasaccharide skeleton **2** in a one-pot yield of 37%. Removal of all ester groups in **2** using Zemplén's deacylation provided the pentaol **24** (84%), which underwent regioselective 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) oxidation of the primary hydroxyl in the D-glucosyl unit to give the carboxylate **25** in 73% yield.[15] Global deprotection by hydrogenolysis finally furnished the target compound **1** in 98% yield. The structure of **1** was confirmed through 1D and 2D NMR and HRMS experiments.

In conclusion, we have developed regioselective one-pot preparations of the requisite monosaccharide building blocks for the synthesis of the tetrasaccharide linkage region of proteoglycans. Stereoselective one-pot glycosidation protocols were also advanced which further simplified the oligosaccharide assembly. The generated linker-attached tetrasaccharide is designed for the examination of the biosynthetic pathway involved in the modification of the sugar units as well as its effects in chain elongation and sorting of GAGs. These explorations will be carried out in the future.

Scheme 6. Synthesis of Compound **1**[a]



[a] BAIB: [bis(acetoxy)iodo]benzene.

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Supporting Information Available. Experimental procedure and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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