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Synthesis of variously sulfated biotinylated oligosaccharides from the linkage region of proteoglycans

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ABSTRACT

The synthesis of a collection, as biotinylated conjugates, of various sulfoforms of the trisaccharide β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*, structures encountered in the linkage region of proteoglycans, is reported herein for the first time. An efficient and stereocontrolled preparation was achieved using common key intermediates in a divergent manner. These molecules should be useful probes to study the substrate specificity of the glycosyltransferases involved at the bifurcation point in the biosynthesis of proteoglycans.

1. Introduction

Proteoglycans (PGs) are biologically important macromolecules composed of glycosaminoglycan (GAG) chains covalently linked to a core protein. They are widespread on the cell surface and in the extracellular matrix, and are involved in many biological processes and pathologies,[1] thus having a great potential as pharmacological targets. PG assembly starts in the Golgi apparatus with the attachment of D-Xylose to a L-serine residue within the protein core. The stepwise addition of two D-Galactose units (D-Gal^{I} and D-Gal^{II}) then one D-Glucuronic acid (D-GlcA) unit provides the so-called common tetrasaccharide intermediate.

At this point, a possible divergence in the biosynthetic pathway may occur (Fig. 1).[2] Transfer of an α-D-GlcNAc residue at O-4 of the terminal non-reducing D-GlcA unit initiates the formation of *gluco*saminoglycans (heparin, heparan sulfate), whereas those of a β-D-GalNAc residue lead to *galacto*saminoglycans (chondroitin sulfate and dermatan sulfate). GAG chains consist of hexosamines and uronic acids arranged in alternating sequences, and these repeating units are variously substituted by sulfate groups creating a great degree of structural and functional diversity. The current hypothesis that the linkage region should be common to all GAG species strongly contrasts with the structural heterogeneity of the GAG region. However, it has been reported that this linkage region may be modified by sulfation at C-4 or C-6 of both D-Gal units, and/or by phosphorylation at C-2 of the D-Xyl unit.[3] The biological significance of these unique substitutions is not yet fully understood, though they could act as biosynthetic signals.[4] Phosphorylation of the D-Xyl unit was demonstrated to be a transient phenomenon involved in the early steps of the biosynthesis.[5,6] Specific sulfation at C-6 of Gal^{I} was found to strongly accelerate the transfer of the D-GlcA unit.[6] But the exact role of these sulfate substituents at the bifurcation of the biosynthesis of PGs has not yet been clarified.

To address this issue, several syntheses of various glycoconjugates of the linkage region containing or not sulfate and/or phosphate groups have been reported,[7] but no clear-cut biological results were obtained with these synthetic molecules. Since phosphorylation or not on the D-Xyl unit apparently has no effect at the divergence level in the biosynthesis, and to shed light on the exact role of the sulfate substituents, we now report for the first time a systematic preparation of all possible sulfoforms of the truncated structure β-D-Glc*p*A-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p* conjugated to biotin to serve as potential substrates for the hexosaminyl transferases involved at the bifurcation point in the biosynthesis of PGs.

2. Results and discussion

For the synthesis of the non-sulfated target molecule **1** and the four possible sulfoforms **2**–**5** (Fig. 2), two common key trisaccharide intermediates **6** and **7** were designed. Diol **6** may be a precursor of **1** and of the two species **2** and **3** sulfated at C-4 and C-6 on Gal^{I}, whereas diol **7** may lead to the two corresponding species **4** and **5** sulfated on Gal^{II}. These later may be in turn prepared by stereoselective assembly of two donors **8** and **9**, which differ in their

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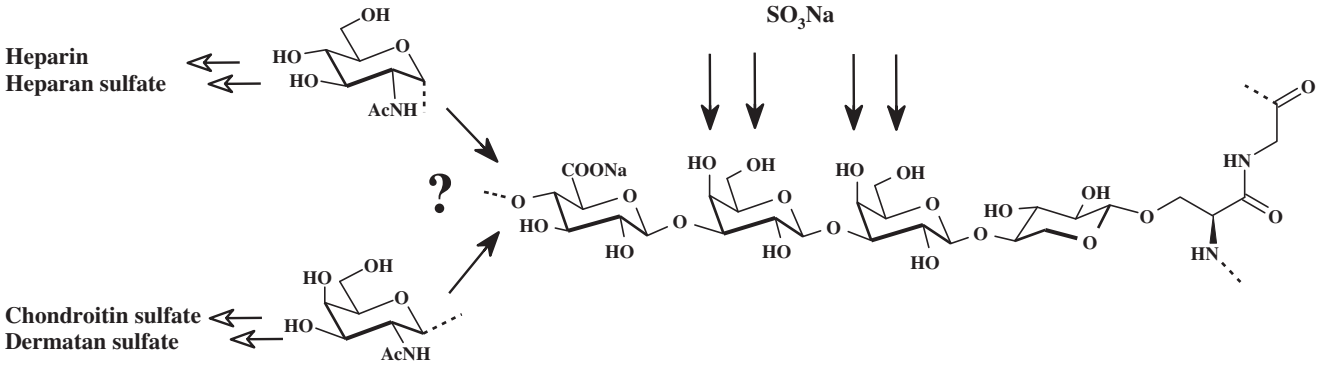

Figure 1. The linkage region of proteoglycans and the bifurcation in their biosynthesis. The arrows indicate possible substitutions with sulfate groups.

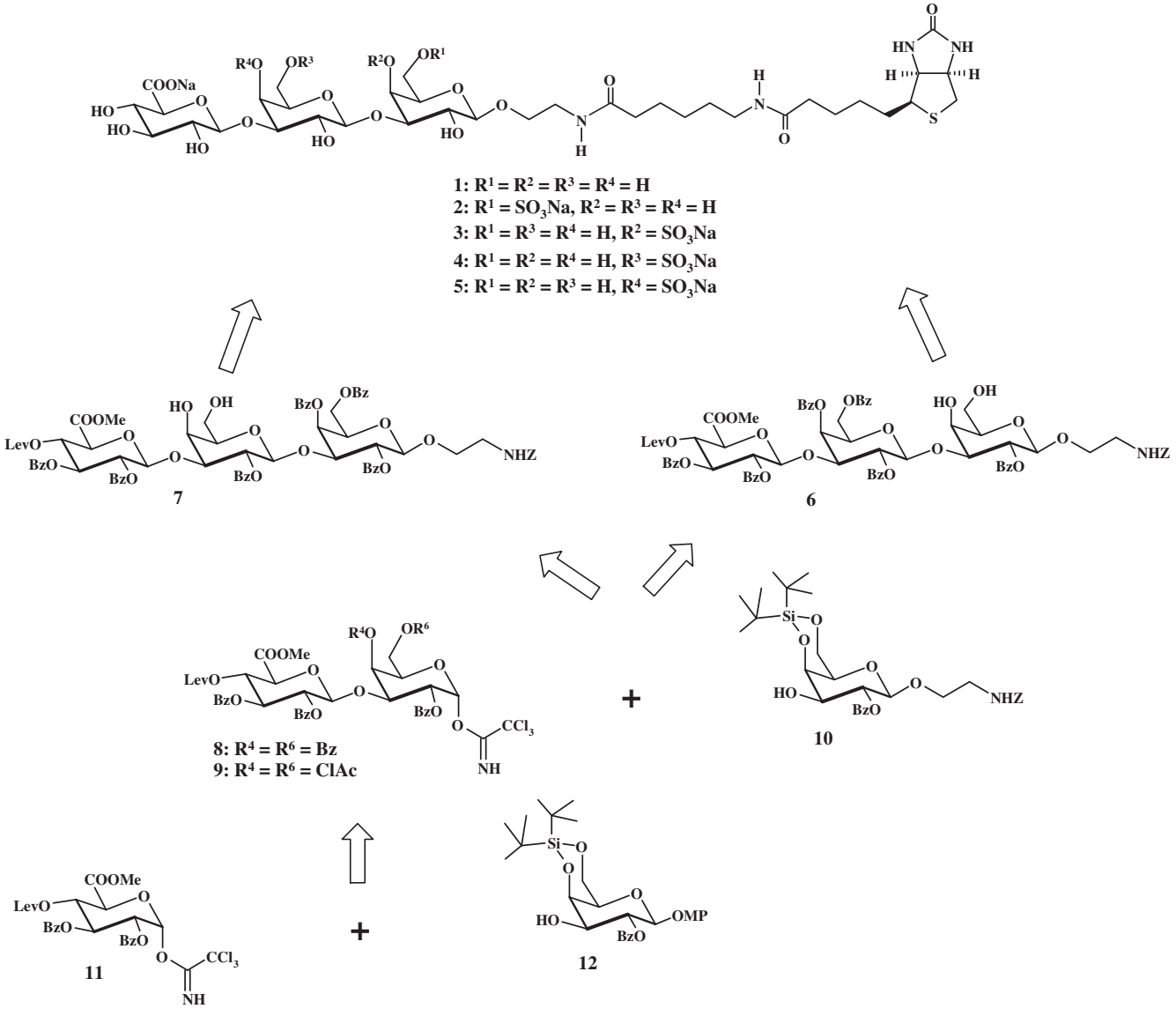

Figure 2. Retrosynthesis for target molecules **1**–**5**.

substitution pattern at C-4 and C-6 of the D-Gal unit, with acceptor **10** still possessing a masked amine linker to allow further coupling with biotin. These two donors may be obtained by stereoselective coupling of donor **11** and acceptor **12**. The β-selectivity in forming all glycosidic linkages would rely on neighboring group assistance by benzoyl groups, which serve also as permanent protective groups. All glycosylation reactions will be performed using Schmidt's trichloroacetimidate procedure.[8] As a prelude for further