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Study on the Systematic Synthesis of Heparan Sulfate Partial Structures

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1. Introduction

Heparan sulfate (HS) is a highly sulfated polysaccharide belonging to glycosaminoglycan (GAG), and commonly found in cell surface and extracellular matrix components. HS is known to regulate biological functions through interactions with various biomolecules, such as growth factors, cell surface receptors, extracellular proteins, and so on. Although HS is basically composed of glucosamine (GlcN)-uronic acid (UroA) disaccharide repeating unit, HS is very heterogeneous because of suffering random enzymatic modification. Recently, a specific structure contained in HS is considered to be responsible for a specific interaction of a target protein, and elucidation of structure-activity relationship of HS becomes an important issue. However, structurally defined HS oligosaccharide is difficult to obtain from the natural source, and its supply by organic synthesis is strongly expected. In this study, novel synthesis of uronic acid moieties, efficient synthesis of HS disaccharide building blocks possessing diverse set for various sugar and sulfation patterns, and interaction analysis between synthesized HS disaccharide structures and HS-binding protein or viruses were examined.

2. Results and Discussions

Two kinds of HS disaccharide building blocks for the construction of HS oligosaccharide library were designed, which is derivable to HS oligosaccharide with various sugar and sulfation patterns. In the synthesis of HS building blocks, preparation of uronic acid moieties, glucuronic and iduronic acid moieties, are key issues. Both of glucuronic and iduronic acid moieties were efficiently prepared from glucrono-6,3-lactone via selective pyranose formation by use of 1,1,3,3-tetraisopropyldisiloxanylidene (TIPS) group. HS disaccharide building blocks were obtained by glycosylation of prepared glucuronic and iduronic acid moieties with glucosamine moiety. Obtained HS disaccharide building blocks were transformed to appropriate sulfated oligosaccharides by means of orthogonal deprotection and sulfation strategy. Conversion of these oligosaccharides to ligand conjugates was achieved by reductive amination reaction of sulfated oligosaccharides.

Sugar chips were then prepared with the synthesized ligand conjugates by the method previously reported. Interaction analysis was performed by surface plasmon resonance (SPR) biosensor, and binding interaction of synthetic HS oligosaccharides and GAG-binding proteins, fibronectin and human recombinant von Willebrand factor (vWF), was examined. The results indicated that binding properties were different depending on HS oligosaccharide structure.

3. Conclusions

HS disaccharide building blocks for HS oligosaccharide precursors have been designed, which possess orthogonally cleavable protective groups and are capable of generating diverse sulfation patterns. Synthesis of uronic acid moieties, which are key issues in the synthesis of HS oligosaccharide, were efficiently prepared using inexpensive glucurono-6,3-lactone as a starting compound. Pyranose formation of uronic acid was selectively achieved using TIDPS group as protecting group. Two disaccharide building blocks were efficiently synthesized using appropriate monosaccharide moieties. Conversion to sulfated oligosaccharides was performed by selective deprotection and sulfation. Interaction analysis of synthesized HS oligosaccharides to appropriate proteins indicated that sugar and sulfation pattern of HS was important for binding.

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