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Toward the assembly of heparin and heparan sulfate oligosaccharide libraries: efficient synthesis of uronic acid and disaccharide building blocks

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ABSTRACT

The monosaccharide moieties found in heparin (HP) and heparan sulfate (HS), glucosamine and two kinds of uronic acids, glucuronic and iduronic acids, were efficiently synthesized by use of glucosamine hydrochloride and glucurono-6,3-lactone as starting compounds. In the synthesis of the disaccharide building block, the key issues of preparation of uronic acids (glucuronic acid and iduronic acid moieties) were achieved in 12 steps and 15 steps, respectively, without cumbersome *C*-6 oxidation. The resulting monosaccharide moieties were utilized to the syntheses of HP/HS disaccharide building blocks possessing glucosamine–glucuronic acid (GlcN–GlcA) or iduronic acid (GlcN–IdoA) sequences. The disaccharide building blocks were also suitable for further modification such as glycosylation, selective deprotection, and sulfation.

1. Introduction

Heparin (HP) and heparan sulfate (HS) are highly sulfated polysaccharides, and are the most complex carbohydrates among the glycosaminoglycan (GAG) superfamily.[1] HP/HS are basically composed of a repeating α or β(1,4)-linked disaccharide unit, which is derived from uronic acid, either glucuronic acid or iduronic acid, and *N*-acetyl-glucosamine residues. The structure is very heterogeneous and contains various substitution patterns derived from the multiple and random enzymatic modifications in their biosynthesis. The diverse micro- or domain structure, which is derived from the enzymatic modification, is considered to regulate the activity of many important biological proteins, such as growth factors, cytokines, viral proteins, and coagulation factors, through their binding interactions in many biological processes.[1,2] The elucidation of the structure–function relations of HP/HS microstructures at the molecular level is, however, very difficult due to their naturally occurring structural diversity. Therefore, structurally defined HP/HS sequences (oligosaccharides) are essential for the precise understanding of the interactions of HP/HS with their target molecules. Many synthetic approaches to the synthesis of HP/HS oligosaccharides have been so far performed.[3–7]

Recently, a synthetic strategy for the assembly of a HP/HS oligosaccharide library has attracted much attention, because it offers a more comprehensive evaluation of HP/HS biological functions.[4c,5,6a] For this accomplishment, the efficient synthesis of uronic acid moieties and disaccharide building blocks accessible to HP/HS oligosaccharides with various sugar and sulfation patterns is required. As neither idose nor iduronic acid derivatives are commercially available, their syntheses are especially important. Currently, iduronic acid moieties are synthesized from glucose or glucuronic acid as a starting material, involving *C*-5 epimerization and/or *C*-6 oxidation.[7] But *C*-5 epimerization sometimes needs to be carried out under harsh conditions, such as, strong basic conditions, and the range of usable protective groups is restricted. *C*-6 Oxidation is also sometimes problematic in yield and repeatability. So far, many intermediates suitable for the structure of HP/HS have been developed as HP/HS building blocks.[3–7]

Previously, we reported the synthesis and analysis of a variety of HP/HS oligosaccharides for investigating their binding properties,[6] in which we clarified that an appropriate HP/HS disaccharide structure was needed for the specific binding between sugar component and protein. Although further investigation of HP/HS binding properties was needed, our previous synthetic route was

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not suitable for the generation of a sufficiently diverse sugar and sulfation pattern. In this study, we synthesized novel disaccharide building blocks for the systematic analysis of HP/HS binding properties.

2. Results and discussion

Our synthetic strategy is shown in Figure 1. The two disaccharide building blocks containing *gluco*- and *ido*-type are prepared from two common monosaccharides, D-glucosamine and D-glucurono-6,3-lactone. They contain a diverse set of protective groups for generating various sulfation patterns. *O*-Sulfation is achieved by selective removal of benzoyl (Bz), 4-methoxybenzyl (MPM), and/or *tert*-butyldiphenylsilyl (TBDPS) groups. Conversion of the azido group to amine, *N*-sulfate, and/or *N*-acetate is achieved by reduction of azido group followed by *N*-sulfation and/or *N*-acetylation. A glucosamine moiety containing different protective groups is easily prepared from glucosamine. A levulinyl (Lev) group at 4-position is introduced for the further elongation of the sugar chain. Two uronic acid moieties are prepared from glucurono-6,3-lactone, which is already oxidized at the *C*-6 position. The synthetic route is expected to be simple because a cumbersome oxidation step can be omitted. Conversion to *ido*-form is achieved by the inversion reaction at *C*-5 position on furanose form.

Syntheses of uronic acid moieties were carried out as shown in Scheme 1. Firstly, inexpensive glucurono-6,3-lactone **1** was transformed to 1,2-isopropylidene **2** according to the method reported previously.[7m] Obtained **2** was then converted to furanose **3** in 43% overall yield in four steps through simple protection and deprotection processes of hydroxy groups. The removal of the isopropylidene group on furanose **3** by treatment with trifluoroacetic acid (TFA) gave triol **4**. Conversion to pyranose form by 1,2-selective protection was then examined. First, 1,2-cyclic acetal protection to obtain pyranose form was used.[4h,7a] Unfortunately, the resulting uronic acid derivatives were a mixture of pyranose and furanose forms under the conditions of acetal formation, suggesting a lower

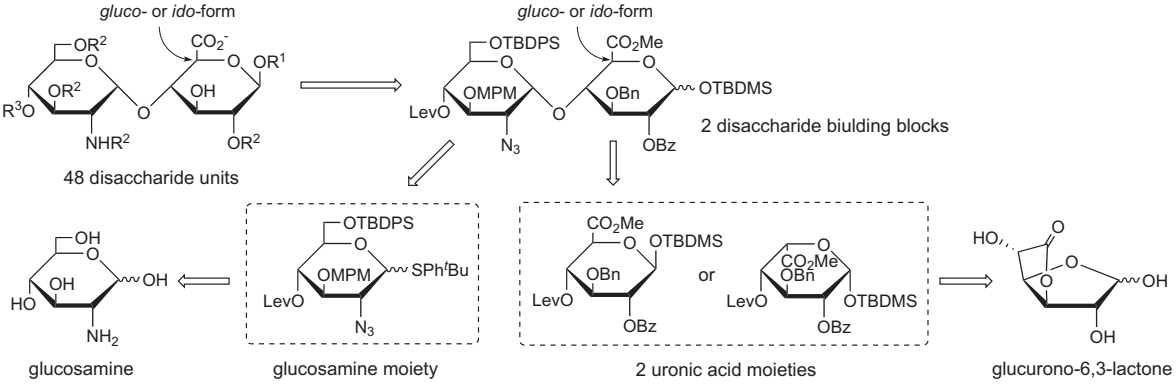
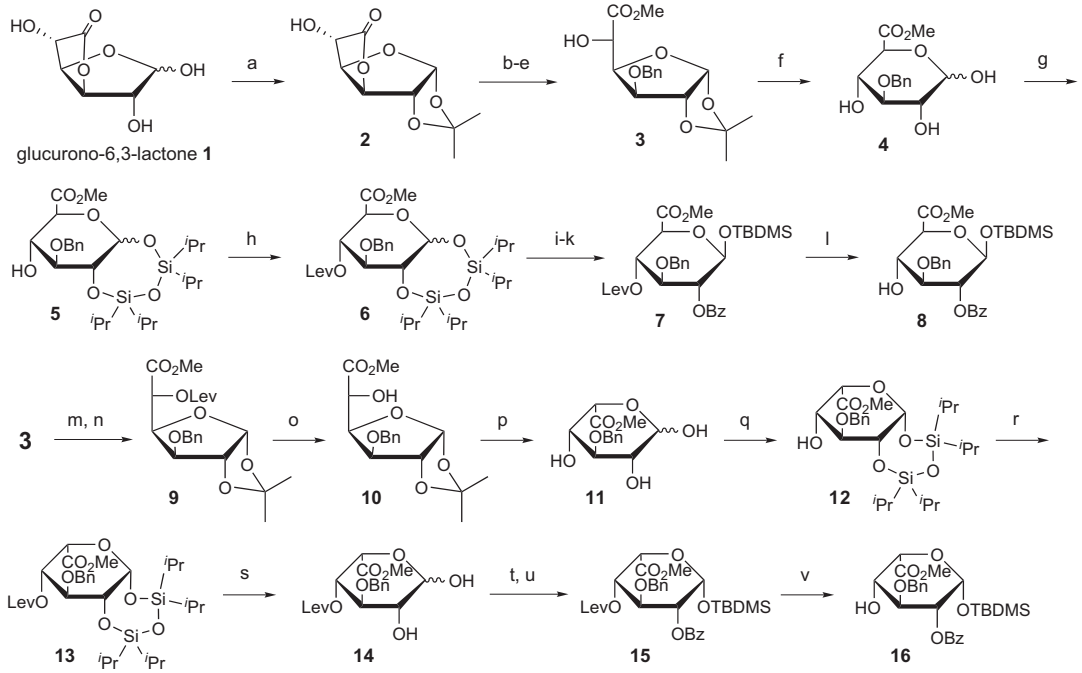


Figure 1. Synthetic route of heparin/heparan sulfate disaccharide structures.



Scheme 1. Syntheses of uronic acid moieties: (a) H_2SO_4 in acetone, 84%; (b) TBDMSCl, imidazole in CH_2Cl_2; (c) 1.0 M MeONa in MeOH (d) $BnOC(=NH)CCl_3$, TBDMSOTf, MS4Ap in CH_2Cl_2, 0 °C; (e) TBAF, AcOH in THF, 43% (four steps); (f) TFA/H_2O (9:1) (83%); (g) $TIPDSCl_2$, imidazole in DMF, 85%; (h) LevOH, EDC·HCl, DMAP in CH_2Cl_2, 84%; (i) TBAF, AcOH in THF; (j) TBDMSCl, imidazole, MS4Ap in CH_2Cl_2; (k) BzCl in pyridine, 78% (3 steps); (l) $H_2NNH_2·H_2O$ in pyridine/AcOH (3:2), 83%; (m) Tf_2O, pyridine in CH_2Cl_2; (n) LevONa in DMF; (o) $H_2NNH_2·H_2O$ in pyridine/AcOH (3:2), 56% (three steps); (p) TFA/H_2O (9:1), 95%; (q) $TIPDSCl_2$, imidazole in CH_3CN, 78%; (r) LevOH, EDC·HCl, DMAP, TEA in DMF, 68%; (s) TBAF, AcOH in THF, 90%; (t) TBDMSCl, imidazole, MS4Ap in CH_2Cl_2; (u) BzCl, DMAP in pyridine, 65% (two steps); (v) $H_2NNH_2·H_2O$ in pyridine/AcOH (3/2), 89%.