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Synthesis of glycosaminoglycan oligosaccharides. Part 4: Synthesis of aza-L-iduronic acid-containing analogs of heparan sulfate oligosaccharides as heparanase inhibitors☆

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ABSTRACT

The synthesis of three azasugar-containing analogs of the disaccharide units of heparan sulfate, which are potential inhibitors of the enzyme heparanase, is reported. Synthetic routes were developed for the preparation of L-*ido*-nojirimycin type glycosyl acceptors with O-4 free. Glycosylation of these acceptors with an O-6 functionalized 2-azido-2-deoxy-D-glucose thioglycoside donor afforded the α-linked disaccharides in good yields. The advantages of using the 4-nitrobenzenesulfonyl group for the protection of the ring nitrogen of azasugars were demonstrated.

1. Introduction

Heparan sulfate proteoglycans (HSPGs) are ubiquitous constituents of the extracellular matrix and of cell membranes.[1] The structure of HSPG consists of a protein core to which linear chains of the glycosaminoglycan, heparan sulfate (HS), are linked by *O*-glycosidic bonds. The carbohydrate backbone of HS is built up of alternating D-glucosamine and hexuronic acid (D-glucuronic acid and L-iduronic acid) units forming →4)-α-L-Ido*p*A-(1→4)-α-D-Glc*p*N-(1→ and →4)-β-D-Glc*p*A-(1→4)-α-D-Glc*p*N-(1→ disaccharides, which are substituted at certain positions. Thus, O-3 and O-6 of the D-glucosamine units, and O-2 of the uronic acid units can be *O*-sulfated, furthermore, the amino group of D-glucosamine can be *N*-acetylated, *N*-sulfated, or remain unsubstituted.[1,2] These variations result in an enormous structural diversity.[3] Heparan sulfate binds to a large number of proteins, thereby influencing a variety of normal and pathological processes including tumor growth and metastasis, tissue repair, angiogenesis, and inflammation.[4] Cleavage of HS chains alters its interaction with proteins and thus influences the above processes.

The most important cleavage enzyme in the catabolism of heparan sulfate chains is heparanase.[5] Heparanase is an *endo*-β-glucuronidase, which specifically cleaves the HS chains at a limited number of sites.[6] It has been recognized that tumor metastasis occurs via complex multistage processes, which involves tumor cell adhesion to various basement membrane components, and degradation of the extracellular matrix and basement membranes. As HS is an important constituent in these structures, cleavage of HS by heparanase plays an important role in cell invasion of some malignant tumors through basement membranes. Heparanase is overexpressed in a number of human tumors.[5b] The expression level of the enzyme is of diagnostic and prognostic value, and has been correlated with the survival time of cancer patients.[5b,7] The inhibition of heparanase forms the basis of potential anti-metastatic cancer therapy, and it has therefore been intensively investigated.[8]

Azasugars are monosaccharide analogs having a nitrogen atom instead of oxygen in the ring, and have received significant attention as carbohydrate mimetics.[9] Compounds of this type, such as 1-deoxynojirimycin, are potent inhibitors of various glycosidases, and are intensely investigated for their therapeutic potential as antidiabetic, antiviral, and anticancer agents. Though monosaccharidic azasugars, in general, show some specificity to inhibit certain types of glycosidases, this specificity is still fairly broad. One way to increase specificity is to use larger size molecules, which are closer mimics of the natural substrates of the enzymes. Thus, oligosaccharides containing an azasugar component have been synthesized for various biological purposes.[10,11]

☆ For Part 3, see Ref. 12.

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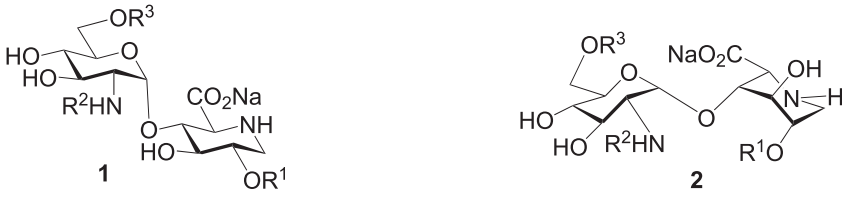
In order to incorporate specificity in azasugars toward heparanase, we have designed azasugar-containing oligosaccharides mimicking the structure of heparin and heparan sulfate (Fig. 1).[12] The synthesis of related aza-analogs of heparan sulfate disaccharides,[13] as well as an interglycosidically *S*-linked oligosaccharide[14] has been reported recently for similar purpose.

2. Results and discussion

2.1. Synthetic design

The target structures **3**–**5** should be available from the 2-azido-2-deoxy-D-glucopyranosyl donor (**7**) and the L-idose

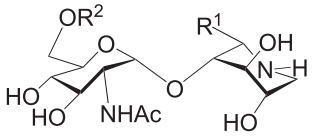


R^1 = H or SO_3Na; R^2 = Ac or SO_3Na; R^3 = H or SO_3Na

Figure 1. Designed azasugar-containing heparan sulfate disaccharides.

Compounds of type **1** and **2** contain a D-glucosamine unit α-(1→4)-linked to an azasugar analog of D-glucuronic acid and L-iduronic acid, respectively. Though heparanase is a β-glucuronidase, compounds of type **2** having an L-*ido*-configured azasugar might also be of interest as potential inhibitors. The rationale for this is that there are several examples reported that azasugars of the 'wrong' configuration are good inhibitors of glycosidases having specificity for a different configuration.[15] Additionally, because of the known conformational mobility of L-idose and L-iduronic acid,[2,16] compounds of type **2** might fit into the active site of the enzyme. It was also of interest, that an L-iduronic acid-type 1-*N*-iminosugar has been reported to have inhibitory activity of cancer metastasis.[17]

Previously only aza-D-glucuronic acid-containing disaccharide inhibitors of heparanase have been reported.[13] As far as we are aware, L-*ido*-configured azasugar-containing oligosaccharides have not been synthesized before, we have recently reported the first compound of this type.[12] We now describe the synthesis of three disaccharides (**3**–**5**) containing azasugar components having L-*ido* configuration, as well as the monosaccharide congener **6** (Fig. 2).



3 R^1 = CH_2OH R^2 = H
4 R^1 = CO_2Na R^2 = H
5 R^1 = CO_2Na R^2 = SO_3Na

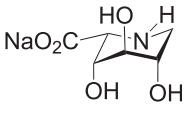


Figure 2. Synthesized azasugar-containing heparan sulfate fragments.

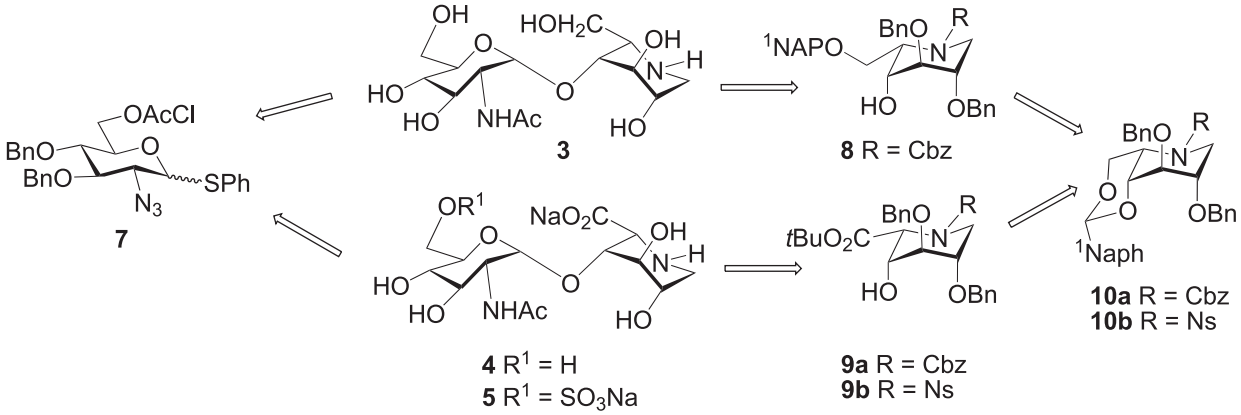
(**8**) and L-iduronic acid (**9**) azasugar glycosyl acceptors (Scheme 1).

In the glycosyl donor **7**, O-6 is masked by the temporary chloroacetyl group, which allows the selective release of O-6 in the synthesis of the *O*-sulfated product (**5**). The carboxyl function in **9** is protected as the *tert*-butyl ester. For the protection of the ring nitrogen in the glycosyl acceptors, the benzyloxycarbonyl group, most commonly used for this purpose in the azasugar field,[10,13] was selected originally. Due to inconveniences and an undesired side reaction recognized during work with the *N*-benzyloxycarbonyl protected derivatives, this group was replaced with the 4-nitrobenzenesulfonyl (Ns) group at a later stage of this work. The synthesis of glycosyl acceptors requires selective manipulations at O-4 and O-6, both **8** and **9** should be available from the (1-naphthyl) methylene acetal **10** by different reductive acetal opening methods giving the readily removable (1-naphthyl)methyl (^{1}NAP) ethers[18] at either O-4 or O-6. The synthesis of the derivatives having L-*ido* configuration was envisioned by S_N2 nucleophilic substitution of *N*-nosyl protected D-*gluco* derivatives in the cyclization step.[19]

2.2. Oligosaccharide synthesis using *N*-benzyloxycarbonyl protection

2.2.1. Synthesis of the glycosyl acceptors. Phenyl 1-thio-β-D-glucopyranoside (**11**), readily obtained by Zemplén deacetylation of the tetraacetate,[20] was converted into the (1-naphthyl)methylene acetal **12**, which was benzylated to give **13** (Scheme 2).

The hemiacetal was released by reaction with NBS[21] and **14** was reduced to give the alditol **15**. The primary hydroxyl was selectively brominated using Ph_3P and CBr_4[22] to give **16** in excellent yield, then



Scheme 1. Retrosynthesis of heparanase inhibitory disaccharides.