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Note

Synthesis of a heparan sulfate mimetic disaccharide with a conformationally locked residue from a common intermediate

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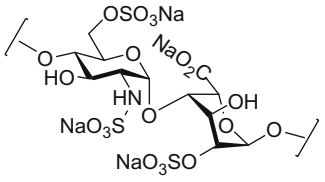
ABSTRACT

A simple mimetic of a heparan sulfate disaccharide sequence that binds to the growth factors FGF-1 and FGF-2 was synthesized by coupling a 2-azido-2-deoxy-D-glucopyranosyl trichloroacetimidate donor with a 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose acceptor. Both the donor and acceptor were obtained from a common intermediate readily obtained from D-glucal. Molecular docking calculations showed that the predicted locations of the disaccharide sulfo groups in the binding site of FGF-1 and FGF-2 are similar to the positions observed for co-crystallized heparin-derived oligosaccharides obtained from published crystal structures.

The fibroblast growth factors FGF-1 and FGF-2 are proteins that play important roles in tumor angiogenesis.[1] They initiate this process by binding with their receptors (FGFRs) and heparan sulfate (HS) to form a ternary complex which leads to receptor dimerization/activation and subsequent cell signaling.[2] Inhibiting the formation of the HS–FGF–FGFR complex by antagonizing HS–FGF binding with HS mimetics is thus a viable strategy for antiangiogenic therapies.[3–5]

Various groups have reported the synthesis of HS and HS-like oligosaccharides designed to interact with FGF-1 or FGF-2.[6–8] These studies have provided valuable information about the structural requirements for oligosaccharide-FGF binding and activation, however, the syntheses of such oligosaccharides are difficult and laborious. This has lead to the pursuit of less synthetically challenging oligosaccharide mimetics as FGF antagonists.[9–12] As part of a program aimed at developing antiangiogenic compounds, we recently described[12] the preparation of simple disaccharides such as **2** and **3** which mimic the HS disaccharide GlcN(2*S*, 6*S*)-IdoA(2*S*) (**1**, Fig. 1), which has been postulated from X-ray crystallographic analyses as a minimal heparin/HS consensus sequence for FGF binding.[13] As well as maintaining the α-(1→4) linkage between the two monosaccharide units and the spatial orientation of the two key sulfo groups [GlcN(2*S*) and IdoA(2*S*)], the compounds were designed to mimic the conformational flexibility[14,15] of the IdoA residue. Docking calculations showed that the predicted locations of disaccharide sulfo groups in the binding site of FGF-1 were consistent with the positions observed for co-crystallized heparin-derived oligosaccharides. Docking scores correlated with experimental K_d values (22 μM to 1.4 mM) obtained from binding assays.[12] The docking score for a model HS disaccharide binding to FGF-1 was similar.[12]

In crystal structures of heparin oligosaccharides bound to FGF, IdoA is found in the $^1C_4$ conformation when bound only to the protein[16,17] or in a skew-boat (^2S_O) conformation when part of a ternary complex.[18,19] NMR studies also indicate that FGF-1 can bind both conformations of IdoA in a bioactive hexasaccharide.[20] These observations led us to consider the synthesis of simple disaccharides in



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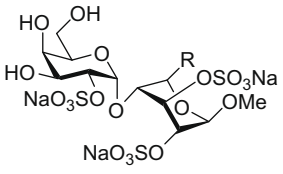

2 R = CH_2OSO_3Na
3 R = H

Figure 1. Structures of the GlcN(2*S*, 6*S*)-IdoA(2*S*) disaccharide sequence **1**, which represents a minimal consensus sequence for FGF–HS binding,[13] and two conformationally flexible mimetics **2** and **3**.[12]

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which the IdoA mimic is locked in the $^1C_4$ conformation. A similar approach has been successfully used to probe the active conformations of the ATIII-binding heparin pentasaccharide.[21,22]

It has been demonstrated that an *O*-sulfo group can substitute for an *N*-sulfo group in a heparin oligosaccharide without loss of binding affinity.[23] The known 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose **8** was thus identified as a suitable IdoA mimic precursor because it is locked in the required $^1C_4$ conformation, can be selectively sulfonated at the C-2 position and is readily available in four steps from D-glucal (**4**) via intermediates **5**–**7** (Fig. 2).[24] The precursor to **8** in this sequence of transformations can, in fact, be easily converted, via intermediates **9**–**11**, into useful glycosyl donors such as the imidate **12** for use in the synthesis of heparin/HS oligosaccharides.[24–26] It was therefore decided to prepare both **8** and **12** and use them to synthesize a disaccharide (**17**) with the desired features.

Following literature precedent,[27] TBDMSOTf was selected as the promoter for the glycosylation of the alcohol **8** with the imidate **12**. The reaction proceeded well in dichloromethane at −20 °C; however, a chromatographically inseparable mixture of anomers resulted (α/β = 3.8:1). The product was thus converted into the tribenzoate via Zémplen deacetylation followed by benzoylation with benzoyl chloride and pyridine, and the desired α-linked disaccharide **13** was isolated by flash chromatography in good overall yield (46%, three steps, Scheme 1), along with 12% of the β-linked disaccharide **13b**. The azide groups of **13** were then reduced via transfer hydrogenation with ammonium formate over $Pd(OH)_2$ catalyst and the resulting diamine **14** was sulfonated with SO_3·trimethylamine complex and debenzoylated (1 M NaOH) to give the benzyl ether **16** in moderate overall yield (28%, three steps). Hydrogenolysis over $Pd(OH)_2$ at 50 psi then furnished the target disaccharide **17** in excellent yield (98%).

Molecular docking calculations were performed using the GLIDE program[28] to examine the binding modes of **16** and **17** with FGF-1 and FGF-2. Compound **16** was examined in order to probe the effects of an extra hydrophobic group on FGF binding as it has been shown that some heparin derivatives with lipophilic modifications can bind to FGF-1 with similar or greater affinity than unmodified heparin.[29,30] The poses of **16** and **17** with the best GlideScores for binding to FGF-1 and FGF-2 are shown in Figure 3. Also shown are the van der Waals surfaces of the central sulfo groups of cocrystallized, heparin-derived hexa- and tetrasaccharide ligands from the crystal structures (pdb accession codes 2AXM[16] for FGF-1 and 1BFB[17] for FGF-2, respectively).

The preferred mode of binding of **16** and **17** to FGF-1 in Figure 3a shows congruence between the ligand sulfo groups with those observed crystallographically. The FGF-2 binding mode of **16**, shown in Figure 3b, also shows the same congruence; however, the preferred mode for **17**, involves ionic hydrogen bonding interactions with the positively charged residues LYS130 and LYS120 of FGF-2. These residues are not involved in binding to the cocrystallized heparin tetrasaccharide fragment, although their proximity to the binding site region and their inherent flexibility suggests that their involvement in ligand binding is reasonable. In the absence

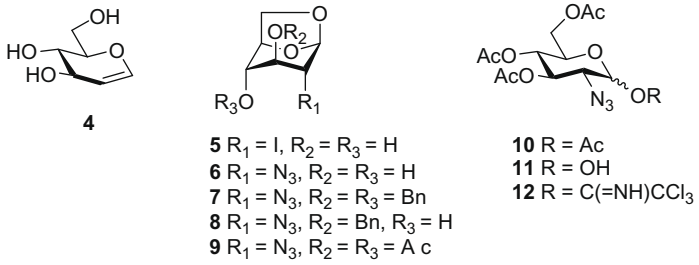
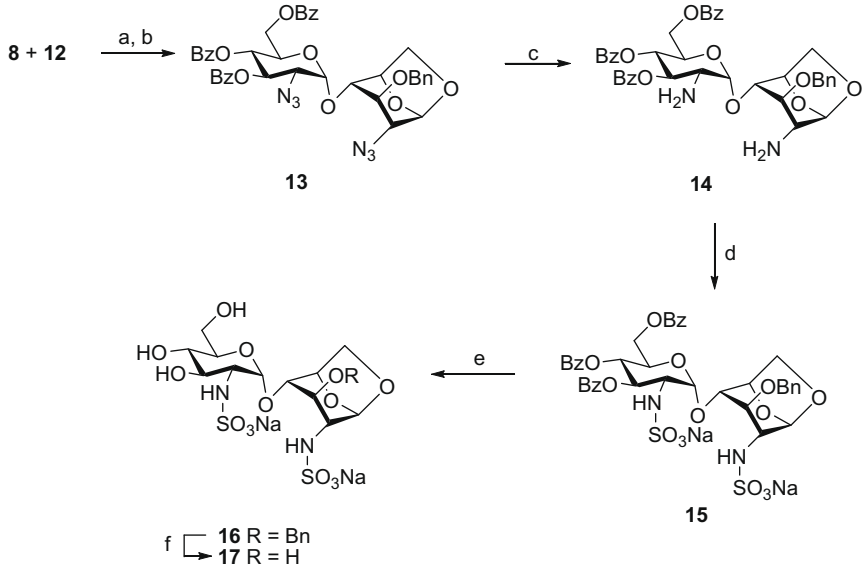


Figure 2. The structures of the glycosyl acceptor and glycosyl donor and their intermediates used in this study.



Scheme 1. Reagents and conditions: (a) TBDMSOTf, CH_2Cl_2, −20 °C; (b) (i) NaOMe, MeOH, (ii) BzCl, pyridine, 46%, three steps; (c) $Pd(OH)_2$, NH_4HCO_2, EtOAc–MeOH, 58%; (d) SO_3·Me_3N, DMF, 60 °C; (e) 1 M NaOH, 28%, three steps; (f) H_2, $Pd(OH)_2$, 98%.