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Design, synthesis, FGF-1 binding, and molecular modeling studies of conformationally flexible heparin mimetic disaccharides

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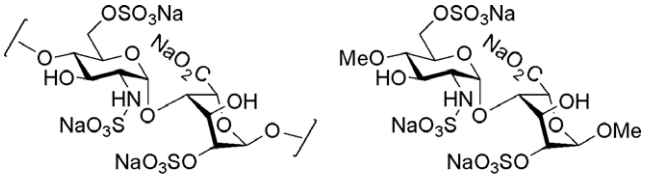
Abstract—Disaccharide mimetics of a heparin sequence that binds to fibroblast growth factors were prepared by coupling a D-galactose donor with a methyl β-D-gluco- or xylopyranoside acceptor. When fully sulfated, the glucose or xylose moieties exist in solution in equilibrium between the $^4C_1$ and $^1C_4$ conformers, as confirmed by ^{1}H NMR spectroscopy, thus mimicking the conformationally flexible L-iduronic acid found in heparin. Docking calculations showed that the predicted locations of disaccharide sulfo groups in the binding site of FGF-1 are consistent with the positions observed for co-crystallized heparin-derived oligosaccharides. Predicted binding affinities are in accord with experimental K_d values obtained from binding assays and are similar to the predicted values for a model heparin disaccharide.

The fibroblast growth factors (FGFs) are a family of structurally related proteins that play important roles in cell proliferation, differentiation, and migration, as well as disease processes such as tumor angiogenesis.[1,2] The most extensively studied members of the FGF family are FGF-1 and FGF-2 which function by binding to and dimerizing a family of signal-transducing FGF receptors (FGFRs), leading to receptor activation and cell signaling. This process is initiated through binding of heparan sulfate (HS) to the FGF and FGFR to form a ternary complex. The manner in which the components of the complex associate is not fully understood, and several models have been proposed.[3–5] Prevention of the formation of the HS:FGF:FGFR ternary complex via blocking the interaction of HS with the FGF may, therefore, form the basis for antiangiogenic therapies.[6–8]

Heparin and HS are glycosaminoglycans (GAGs) composed of repeating 1 → 4 linked disaccharide sequences of α-D-glucosamine (GlcN) and a uronic acid (β-D-glucuronic acid, GlcA, or α-L-iduronic acid, IdoA).[9] Short heparin/HS sequences (di- and trisaccharides[10] and tetrasaccharides[11]) bind to the FGFs, although more recent studies indicate that unsulfated di- and trisaccharides do not bind FGF,[12] and longer oligosaccharides are usually required for the promotion of dimerization and activation. X-ray crystal structures of heparin-derived oligosaccharides bound to FGFs alone[13,14] or in ternary complex with FGFR[15,16] have been determined. Analyses of these structures reveal that not all oligosaccharide sulfo and carboxyl groups bind to FGF, and that the disaccharide GlcN(2*S*,6*S*)-IdoA(2*S*) (**1**, Fig. 1) represents a minimal heparin/HS consensus sequence for FGF binding.[17] The 2-*O*-sulfo group of IdoA and the *N*-sulfo group of GlcN in **1** form the primary protein contacts and in the case of FGF-1,



1 2

Figure 1. Structure of the GlcN(2*S*,6*S*)-IdoA(2*S*) disaccharide sequence **1**, which represents a minimal consensus sequence for FGF:HS binding,[17] and a model disaccharide **2** considered in the theoretical calculations presented here.

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the GlcN 6-*O*-sulfo group also interacts favorably with the protein.[17] In these structures the GlcN residue of **1** adopts the normal $^4C_1$ chair conformation whilst the conformationally flexible[18] IdoA is found in the $^1C_4$ conformation when bound only to the protein[13,14] or in a skew-boat (^2S_O) conformation when part of a ternary complex.[15,16] Recent NMR studies indicate that FGF-1 can bind both conformations of IdoA in a bioactive hexasaccharide.[19]

A number of structurally simple heparin mimetics also bind to the FGFs in the HS binding site and inhibit FGF mitogenic activity, for example, sulfonated naphthalenes[20] and sulfated oligosaccharides[6,21] and their glycosides.[22,23] Taken together, these observations lead us to conclude that structural mimics of disaccharide **1** might be profitably explored as potential inhibitors of FGF-mediated angiogenesis.

The synthesis of heparin/HS oligosaccharides is challenging,[24] and syntheses of 'non-regular' disaccharides, that is, with GlcN at the non-reducing end, have only been reported in the past decade. Despite recent progress,[25–27] a major hurdle in this area continues to be synthetic access to IdoA. Simple disaccharides, therefore, were sought that could be readily synthesized while mimicking the essential features of **1**, namely the α-(1 → 4) linkage between the two monosaccharide units, the spatial orientation of the two key sulfo groups [GlcN(2*S*) and IdoA(2*S*)], and the conformational flexibility of the IdoA residue. It was considered that suitable disaccharides might be synthesized by glycosylation of an IdoA mimic with a differentially 2-*O*-protected glycosyl donor.

The D-thiogalactosides **3** and **4** were chosen as glycosyl donors (Fig. 2) because of the ease with which the 2-OH can be differentially protected. It was assumed that *N*-sulfo groups could be substituted for by *O*-sulfo, as previously demonstrated with analogues of the AT III-binding heparin pentasaccharide.[28] It was also hypothesized that D-galactose (Gal) in the place of GlcN at the non-reducing end would not significantly reduce FGF binding. The β-configuration and the non-participating 2-*O*-benzyl group were expected to favor the stereoselective formation of the desired α-(1 → 4) linkage. Like the naturally occurring GlcN, the Gal residue should adopt a $^4C_1$ conformation in solution. Importantly, the protecting group strategy also permits the other hydroxyl groups of this residue to remain either unprotected or derivatized as non-polar methyl ethers[28] which allows for the testing of small hydrophobic groups in FGF ligand binding. Glycosyl donor **4** allows for sulfonation at *O*-6 enabling examination of the effects of the 6-*O*-sulfo group that is present in **1**.

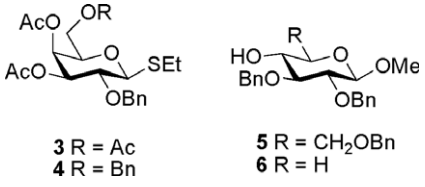

Figure 2. Structures of glycosyl donors (**3** and **4**) and acceptors (**5** and **6**) used in this study.

The selection criteria for IdoA mimics as glycosyl acceptors included ease of synthesis and ability to adopt the $^1C_4$ conformation in solution, thus presenting a suitably orientated 2′-*O*-sulfo group for binding to FGF. Highly sulfated β-D-gluco- and -xylopyranosides are known to exist in a conformational equilibrium between the two chair conformers in favor of the $^1C_4$.[29,30] The tribenzyl β-D-glucoside **5** and dibenzyl β-D-xyloside **6** were thus selected as glycosyl acceptors because, once deprotected and sulfonated, the glucose or xylose ring should exhibit the desired conformational flexibility. It was hypothesized that the additional sulfates in the ring beyond that required at *O*-2′ would not unduly interfere with binding.

The Gal-Glc disaccharides were prepared as shown in Scheme 1. The acceptor alcohol **5**[31] was glycosylated at −20 °C with thioglycoside donor **3**,[32] using *N*-iodosuccinimide/triflic acid (NIS/TfOH) as promoter, to give a 4:1 α/β mixture of anomers from which pure disaccharide **7** was obtained by flash chromatography in satisfactory yield (61%).[33] Hydrogenolysis of the benzyl ethers (H_2/Pd(OH)$_2$ on C) proceeded in good yield (91%) and subsequent sulfonation (sulfur trioxide trimethylamine complex) and deacetylation (1 M NaOH) gave the desired tetrasulfate **13**, which was purified by size exclusion chromatography (Bio-Gel P-2). The purity (⩾96%) was determined by capillary electrophoresis (CE)[34] and NMR spectroscopy. The trimethylated derivative **11** was prepared by quantitative deacetylation and methylation (NaOMe/MeOH and NaH/MeI) of **7**. Hydrogenolysis of the benzyl ether protecting groups (H_2/Pd on C) gave tetrol **16** in good yield (79%), and subsequent sulfonation afforded trimethylated tetrasulfate **17**. The corresponding pentasulfates **14** and **18** were prepared analogously starting from glycosyl donor **4**.[35]

For the synthesis of the xylose analogue of **13** (Scheme 2), glycosylation of xylose acceptor **6**[36] with donor **3** gave an inseparable mixture of anomers (α/β = 5:1) that was converted into the corresponding mixture of tribenzoates from which the pure α-anomer **19** was obtained by flash chromatography. Hydrogenolysis to give triol **20**, followed by sulfonation/debenzoylation, afforded the trisulfate **21** in moderate yield (24%, three steps from **19**).

Purification of the sulfated disaccharides was challenging and the final products were obtained in low yields (3–24%), in part, due to contamination by inorganic salts and the presence of undersulfated species which were difficult to separate chromatographically. The magnitude of the vicinal coupling constants observed in the ^{1}H NMR spectra indicates that the Glc rings of **13**, **14**, **17**, and **18**, and the D-xylose ring of **21**, exist in a chair–chair equilibrium in favor of the $^1C_4$ chair conformation (Table 1), as anticipated.[29,30]

The binding affinities of the sulfated disaccharides **13**, **14**, **17**, **18**, and **21** for FGF-1, measured using a surface