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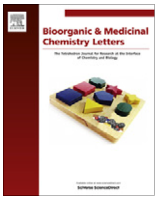

A focused sulfated glycoconjugate Ugi library for probing heparan sulfate-binding angiogenic growth factors

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ABSTRACT

A library of small molecule heparan sulfate (HS) mimetics was synthesized by employing the Ugi four-component condensation of D-mannopyranoside-derived isocyanides with formaldehyde as the carbonyl component and a selection of carboxylic acids and amines, followed by sulfonation. The library was used to probe the subtle differences surrounding the ionic binding sites of three HS-binding angiogenic growth factors (FGF-1, FGF-2 and VEGF). Each compound features 3 or 4 sulfo groups which serve to anchor the ligand to the HS-binding site of the protein, with a diverse array of functionality in place extending from C-1 or C-6 to probe for adjacent favorable binding interactions. Selectivity of binding to these proteins was clearly observed and supported by molecular docking calculations.

Finding more efficient approaches to accelerate lead discovery and optimization has been a continuing theme for drug discovery in recent years. Out of many outstanding strategies, multicomponent reactions have attracted significant attention from both the academic and industrial sectors due to their efficiency in the generation of chemical diversity.[1] The Ugi four-component condensation is the most important isocyanide-mediated multicomponent reaction, and has been explored in many drug discovery and development applications[2] including in the glycomics area.[3] Some years ago the potential of the Ugi reaction for targeting angiogenic heparan sulfate (HS)-binding growth factors such as fibroblast growth factors 1 and 2 (FGF-1 and -2) and vascular endothelial growth factor (VEGF) was demonstrated.[4,5] Herein we report a focused library approach utilizing the Ugi reaction for probing the ligand–protein binding sites in order to identify higher affinity small molecule leads for these HS-binding growth factors.

The FGFs and VEGF are important mediators of angiogenesis and thus are attractive targets for drug discovery.[6] They initiate cell signaling cascades that lead to angiogenesis by initial formation of ternary complexes with HS and their cognate receptors. Inhibiting angiogenesis by targeting the HS-binding site with HS mimetics and thus blocking the formation of these ternary complexes is a viable therapeutic strategy for cancer.[7] The FGFs have been widely studied and a number of X-ray crystal structures are available, including in complex with heparin-derived oligosaccharides,[8] and in ternary complex with its receptor (FGFR) and a heparin-derived oligosaccharide.[9] The HS-binding sites of these proteins share a common feature, that is, a densely charged, cationic, shallow groove.

Over the past decade, we have explored the use of small molecule HS mimetics as potential inhibitors of FGF- and/or VEGF-mediated angiogenesis for the development of novel cancer therapeutics.[10] Such approaches avoid the inherent synthetic complexity associated with natural HS oligosaccharide sequences and have the potential for modifying biological activity by simple chemical modifications. Among the various approaches, we reported[10e] the use of a panel of linked sulfated cyclitols containing the core structure **3** (Fig. 1) to probe various HS-binding proteins. These studies indicated that ligand selectivity towards such proteins could be influenced by subtle changes in the size and orientation of the spacers between the two highly charged centres, which is in agreement with the generally accepted view that different domains or structural motifs of HS bind to different proteins to mediate their effects.[11]

In the current design strategy a simple ionic binding motif on a monosaccharide scaffold that could act to ‘anchor’ the ligand to the HS-binding site on the target protein was envisaged. The Ugi reaction would then be utilized to decorate the scaffold, via various linkers, with a diverse range of functional groups in order to probe for additional favorable binding interactions. This concept allows for the rapid assembly of a library of small molecules for use as a tool to map the structural tolerances on the surrounding surface of the ionic binding site of these related proteins, with the aim of

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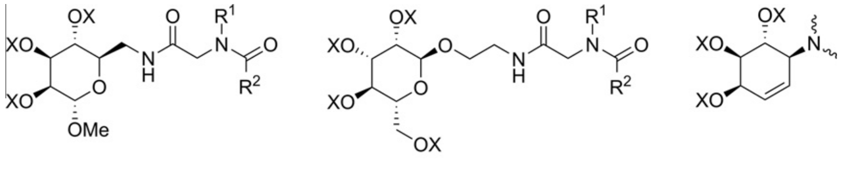


1 2 3

Figure 1. Library design based on Man-6-isocyanide (**1**; series 1) and Man-1-isocyanide (**2**; series 2) and the core structure motif of linked cyclitols (**3**). X = SO_3Na.

improving affinity and selectivity for each individual protein. This approach also seeks to reduce the overall charge of the ligands by minimizing the number of sulfo groups in the HS-binding anchor and by introducing mostly hydrophobic (aliphatic or aromatic) groups via the Ugi reaction.

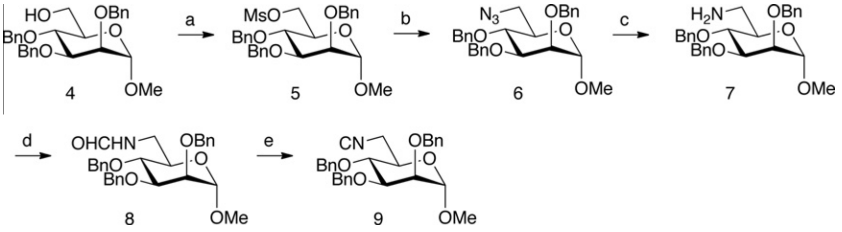
To limit the number of possible structures, we selected only two monosaccharide scaffolds **1** and **2** based on D-mannopyranose with extension from the more readily accessible positions C-1 and C-6 (Fig. 1). These common scaffolds were designed to contain the isocyanide component for the Ugi reaction whilst the carboxylic acid and amine components could be selected from a vast range of commercially available structures. The simplest carbonyl component, formaldehyde, was chosen as the fourth partner to avoid the complexity of generating an additional diastereomeric centre. The selection of D-mannose was also based on existing internal programs involving this sugar as well as the presence of a pair of *cis*-oriented vicinal hydroxyl groups which can be sulfonated, a feature common to the linked cyclitol core structure **3**.

The preparation of mannoside-6-isocyanide **9** is outlined in Scheme 1. By following standard functional group transformations, the alcohol **4**[12] was converted into the corresponding amine **7** in 54% overall yield. Subsequent formylation and dehydration afforded **9** in high yield (93%). The corresponding C1-isocyanide **10** was prepared as reported earlier.[4] The structures of the intermediates and products were characterized by ^{1}H and/or ^{13}C NMR spectroscopy.[13] Both building blocks **9** and **10** showed characteristic signals for isocyanide carbon at 157.63 and 157.57 ppm, respectively, in ^{13}C NMR spectra.

The components for the Ugi library construction are illustrated in Scheme 2. Although formaldehyde has not been used frequently in the Ugi reaction, we found in general that the yields using this carbonyl source were good. In a few cases, elevated temperatures were required to increase the yield (such as entry 9, Table 1). The presence of free hydroxyl groups was generally well tolerated during the Ugi reaction except in the case of glycolic acid, which gave an unidentified mixture. However, protection of the hydroxyl group as the benzyl ether gave the desired Ugi adduct in good yield (69%, ∗∗∗entry 13, Table 1). Global debenzylation was carried out in quantitative yield by hydrogenolysis with 20% $Pd(OH)_2$ on charcoal as catalyst. The crude polyol, after drying under high vacuum in the presence of phosphorus pentoxide, was used directly for the sulfonation step. Purification of the multi-charged products was not always straightforward and sometimes required a combination of several purification techniques, such as silica gel or size-exclusion column chromatography and solid phase extraction (SPE). For those compounds with an extra carboxylate group, which was introduced as the methyl ester during the Ugi reaction step, the crude sulfated product was further subjected to a sodium hydroxide mediated hydrolysis step prior to the standard purification. The purified product fractions were individually analysed by capillary electrophoresis (CE), pooled and lyophilized to give the final sulfated material as an amorphous powder (purity $\geqslant$ 98% by CE).

The binding affinities of the library for FGF-(1,2) and VEGF were measured using a surface plasmon resonance (SPR) solution affinity assay (Table 1).[14] The affinities for the non-Ugi elaborated parent compounds[10f,15] trisulfate **15** and tetrasulfate **16** are also provided for comparison. The measured K_d values clearly showed the varied preference of each protein towards the ligands, with the affinities spanning 38-, 7- and 104-fold differences for FGF-1, FGF-2 and VEGF, respectively. For FGF-1, either an aromatic group or an extra negative charge is preferred to an aliphatic group for increased affinity. Additional charged groups either in the D-mannoside ring or as an attached functional group did not always correlate with improved affinity. For example, **1Aa**-**1Ba**-**1Ca**-**1Ig**-**1Ih**-**1Ii** showed lower affinities compared with their corresponding series 2 compounds. **1He** (37 μM) not only binds more tightly than its Series 2 analogue **2He** (171 μM) but is also one of the best compounds out of the panel for FGF-1. Although extra charged groups (motifs g, h, i) had a greater influence when delivered in Series 2 (20–44 μM) compared with Series 1 (111–338 μM), it is aromatic groups that result in the tightest binding compound (**2Ca** at 11.8 μM) and provide more useful SAR information (the homologues **2Ba** and **2Aa** at 24.8 and 97.4 μM, respectively). The affinities of the ligands towards FGF-2 showed the smallest variation, with the best two containing either a flexible aromatic group (113 μM for **2Ca**) or an extra negative charge (118 μM for **2Ig**).

For VEGF, ligand binding affinities followed a similar trend to FGF-1, which may imply that there is some similarity in their HS-binding domains. However, one striking difference was the intolerance of VEGF to cyclic aliphatic rings (1–3 mM for D, E, F, G), while an aromatic group (28.8–109 μM for motif A, B, C, d, e)



Scheme 1. Reagents and conditions: (a) MsCl (1.5 equiv), DMAP (0.11 equiv), Et_3N (3 equiv), DCM (0.06 M), 0–18 °C, 18 h, 100%; (b) NaN_3 (5 equiv), DMF (0.2 M), 90 °C, 3 h, 63%; (c) (i) PPh_3 (1.3 equiv), THF (0.2 M), 18 °C, 1 h, (ii) H_2O (20 equiv), 18 °C, 18 h, 85%; (d) Ac_2O (30 equiv), HCO_2H (0.06 M), 18 °C, 3.5 h; (e) $POCl_3$ (2 equiv), TEA (4 equiv), DCM (0.05 M), 0 °C, 2 h, 93% (two steps).