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Glycosidase Inhibition by Multivalent Presentation of Heparan Sulfate Saccharides on Bottlebrush Polymers

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Abstract

We report herein the first-time exploration of the attachment of well-defined saccharide units onto a synthetic polymer backbone for the inhibition of a glycosidase. More specifically, glycopolymers endowed with heparan sulfate (HS) disaccharides were established to inhibit the glycosidase, heparanase, with an IC_{50} value in the low nanomolar range (1.05 ± 0.02 nM), a thousand-fold amplification over its monovalent counterpart. The monomeric moieties of these glycopolymers were designed in silico to manipulate the well-established glycotope of heparanase into an inhitope. Studies concluded that (1) the glycopolymers are hydrolytic stable toward heparanase, (2) longer polymer length provides greater inhibition, and (3) increased local saccharide density (monoantennary vs diantennary) is negligible due to hindered active site of heparanase. Furthermore, HS oligosaccharide and polysaccharide controls illustrate the enhanced potency of a multivalent scaffold. Overall, the results on these studies of the multivalent presentation of saccharides on bottlebrush polymers serve as the platform for the design of potent glycosidase inhibitors and have potential to be applied to other HS-degrading proteins.

Graphical Abstract

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Notes

The authors declare no competing financial interest.

Supporting Information

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INTRODUCTION

Protein–saccharide binding events mediate a myriad of important biological functions including cell–cell adhesion, cell growth, and immune defense.^{1,2} Interestingly though, individual saccharide–lectin binding events are relatively weak, and with association constants around 10^6 M^{-1} are not sufficient to control in vivo events.^{3–5} Nature’s way of circumventing these limitations is through multivalency.^{2,4–8} Placement of multiple identical weak-binding monomeric saccharide ligands in high density to one another increases binding specificity as well as induces more favorable overall entropy and binding enthalpy.^{4,6} The enhancement is attributed to the increased number and cooperativity of possible binding events and is termed “the cluster glycoside effect”.⁴

Natural polysaccharides are multivalent ligands that can serve as both potent inhibitors and effectors of biological processes, which have made them highly desirable medicinal targets.⁹ However, isolation of polysaccharides from natural sources can result in microheterogeneity and limited supplies. Furthermore, chemical synthesis of these natural polysaccharides is a daunting and insurmountable task. As a result, recent efforts have employed the attachment of low molecular weight and well-defined glycotopes onto synthetic multivalent scaffolds as mimetics of native polysaccharides.^{2,5–10} These synthetic multivalent sugar-functionalized ligands are facile to prepare and have been illustrated to preserve the inhibitory and signal transduction properties of the natural polysaccharides.² The architecture of scaffolds used for glycoclustering are wide ranging from dendrimers,¹¹ polymers,^{2,5–10} cyclodextrins,¹² calixarenes,¹³ nanoparticles,¹⁴ peptides,¹⁵ to quantum dots.¹⁶ Out of these multivalent motifs, linear glycopolymers were the first and have been the most widely studied in their interactions due to their ability to be easily manipulated and controlled; including saccharide composition and density, variation in linker (length, configuration, and flexibility), and polymer length.^{2,5–10} In particular, the use of ring-opening metathesis polymerization (ROMP)-based scaffolds have shown great promise for glycopolymers.^{17,18}

Traditionally, most studies using multivalent saccharide containing scaffolds have been conducted on lectins such as concanavalin A.^{2,3} In comparison, glycosidases and other carbohydrate-processing enzymes are generally monomeric, binding to only a single oligosaccharide sequence with high affinity and specificity. In addition, many glycosidases possess deep catalytic sites. These principles have deterred the use of multivalent inhibiting motifs with the focus being toward use of transition state mimicking monovalent compounds, such as iminosugars. Nevertheless, recent studies have shown that multivalency can amplify the inhibition of carbohydrate-processing enzymes.^{19–30} For instance, fullerenes,^{21–23} nanoparticles,²⁴ cyclodextrins,^{20,25–28} and nanodiamonds,²⁹ with the appropriate pendant carbohydrate motif, have been utilized in the inhibition of various glycosidases. However, to the best of our knowledge, the use of synthetic polymers have yet to be fully explored.^{19,20,30}

Glycosidases represent an abundant and important constituent of enzymes that largely remain underdeveloped as powerful medicinal targets. One such potential enzyme is the β -endoglucuronidase, heparanase, whose main function is to degrade internal GlcA β (1,4)GlcN glycosidic bonds of heparan sulfate (HS) proteoglycans (**1**, Figure 1) in the extracellular

matrix.^{31,32} The enzyme uses two highly positively charged domains (HBD-1 and HBD-2) to guide heparan sulfate chains into the hydrolytic active site between the two domains.³³ The recognized motif to be cleaved is highly specific to the sulfation pattern of the HS chains (**1**).³⁴ The degradation of the HS chains has illustrated heparanase to be the regulator of aggressive tumor behavior as well as to play key roles in the progression of kidney related diseases and autoimmune diabetes.^{31,32,35–37} As such, heparanase inhibition has become a highly pursued clinical target, with the most potent molecules being HS carbohydrate-based mimetics.²

Currently, these HS carbohydrate-based mimetics are sulfated oligo- and polysaccharides isolated from natural sources, which were then slightly modified to increase affinity to heparanase.^{31,32} We envisioned that employing the use of neo-glycopolymers as HS mimics would allow for homogeneous molecules, while having control over the aforementioned properties to increase the binding affinity to heparanase. Most importantly, this approach provides the flexibility to control the sulfation pattern, minimizing the potential for deleterious crossbioactivity. Previous HS neo-glycopolymer mimics consist of simple persulfated glucosamine or lactose.^{38–42} Recently, the Hsieh-Wilson group was able to show that by controlling the sulfation pattern of the pendant saccharide and replicating the glycotope of the natural glycosaminoglycan substrate, it was now capable of selectively replicating the signaling event.^{43–45}

Heparan sulfate (HS) polysaccharides (**1**) are comprised of linear, repeating [D-HexUA(β 1,4)D-GlcNX(α 1,4)]_n disaccharide units, where HexUA can either be glucuronic acid (GlcA) or its C(5) epimer, iduronic acid (IdoA; Figure 1). These HexUA are linked to either a *N*-sulfoglucosamine (GlcNS) or a *N*-acetylglucosamine (GlcNAc) unit.⁴⁶ The heterogeneity of HS allows it to bind to a large number of lectins for mediation of various biological functions. Therefore, it is of utmost importance that the proper sulfation pattern is chosen when targeting a specific GAGs binding protein.⁴⁶ Herein, we describe the design and synthesis of novel multivalent glycopolymer inhibitors of the glycosaminoglycan degrading glycosidase enzyme, heparanase. We also further investigate the effect of increasing the local saccharide density on the inhibition of heparanase by comparing inhibitory concentrations of diantennary monomer and its corresponding glycopolymer **3** (Figure 1) to those for monoantennary monomer and its corresponding glycopolymer **2**. This approach could potentially be utilized as a platform to design glycopolymers with a well-defined sulfation pattern for inhibiting other GAGs degrading enzymes by tailoring to the specific enzyme's binding epitope.

EXPERIMENTAL SECTION

General Procedure for Polymerization

A 1 mL solution of monomer in a degassed mixture of 1,2-dichloroethane/2,2,2-trifluoroethanol (DCE/TFE = 2.5:1) was transferred to an oven-dried 10 mL Schlenk flask under N₂ (note: solvent mixture was degassed by freeze–pump–thaw method and repeated at least five times until bubbles subsided). The mixture was then concentrated by rotary evaporation and placed in vacuo for 30 min. In a glovebox under an inert N₂ atmosphere, a conical 1 mL oven-dried Schlenk was charged with third generation Grubbs catalyst **24**

[(H₂IMes)(3-Br-py)₂(Cl)₂Ru=CHPh] (Table 1), then sealed with a glass stopper and removed from the glovebox. The catalyst **24** was then dissolved in the degassed 2.5:1 DCE/TFE mixture under N₂ to make a stock solution. Under N₂, monomer was redissolved in the degassed 2.5:1 DCE/TFE mixture, and 0.1 mL of the catalyst **24** stock solution was then rapidly injected to the monomer solution. The resulting mixture was sealed with a glass stopper (final concentration = 0.025 M) and allowed to stir at 55 °C. After the solution became cloudy (1 h), the conversion of the monomer was monitored by ¹H NMR of a reaction aliquot in CD₃OD by observing the disappearance of the strained alkene peak at 6.4 ppm. Upon full conversion, the reaction mixture was cooled to room temperature and stirred for 5 min. The mixture was then quenched with ethyl vinyl ether (10 drops) and allowed to stir for an additional 30 min. After the reaction mixture was transferred into a 20 mL scintillation vial, it was concentrated in vacuo to a brown oil. The crude product was dissolved in a minimal amount of methanol and precipitated with an excess of diethyl ether. Precipitate was allowed to settle and the liquid was then decanted off. Note: If the precipitant was very fine, this solution was centrifuged, and the diethyl ether layer was decanted. The precipitate was then redissolved in excess methanol (2 mL) and reconcentrated until the polymer was in a minimal amount of methanol. This process was repeated two more times. On the final precipitation, the polymer was not redissolved in methanol and placed in vacuo to yield the glycopolymer as an off white solid. Polymers of same scaffold showed no variation in ¹H NMR signals, only varying in the ratio of the GlcN anomeric peak (~5.5 ppm) and the phenyl end group (~7.4 ppm), which were used to find the DP (note: since the resulting polymers are prone to form micelles and could not be characterized by GPC, the ¹H NMR end group analysis was used to determine their DP).

Critical Micelle Concentration (CMC)⁴⁷

Fluorescence measurements were performed in an Aligent Technologies Cary Eclipse Fluorescence Spectrophotometer. A 15 μM stock solution of pyrene was generated in a 15:85 methanol/water mixture. A stock solution of polymer was serially diluted in 1.5 mL Eppendorf tubes to a volume of 420 μL at 16 different concentrations with deionized water from 0 to 1 mg/mL. To each tube of polymer solution, 30 μL of the pyrene stock solution were added to bring the final pyrene concentration to 1 μM and a methanol concentration of <1%. These tubes containing the polymer and pyrene solution were then covered in aluminum foil and mechanically agitated by an orbital shaker for 2 h and then allowed to equilibrate for 18 h. Fluorescence emission spectra of the polymer solutions containing pyrene were recorded in a 400 μL microcuvette with an excitation wavelength of 335 nm, and the emission intensities *I*₁ and *I*₃ were measured at the wavelengths corresponding to the first and third vibronic bands located near 373 (*I*₁) and 384 (*I*₃) nm. A 2.5 nm slit width was used for both excitation and emission. All fluorescence measurements were carried out at room temperature. The average ratio of *I*₁/*I*₃ for three trials was plotted against the concentration of each polymeric sample using GraphPad Prism 7. The CMC was determined at the intersection of two calculated regression lines.

TR-FRET Heparanase Inhibition Assay⁴⁸

A total of 42 μL of inhibitor solution in Milli-Q water (0.00016–4000 μM) or just Milli-Q water (as a control) and 42 μL of heparanase (5.3 nM, R&D Systems) solution in pH 7.5 tris

buffer (consisting of 20 mM Tris HCl, 0.15 M NaCl, and 0.1% CHAPS) or just buffer as a blank were added into microtubes and preincubated at 37 °C for 10 min. Next, 84 μ L of biotin-heparan sulfate-Eu cryptate (Cisbio, Cat # 61BHSKAA; 58.6 ng in pH 5.5 0.2 M NaCH₃CO₂ buffer) was added to the microtubes, and the resulting mixture was incubated for 60 min at 37 °C. The reaction mixture was stopped by adding 168 μ L of Streptavidin-XLent! (Cisbio, Cat # 611SAXLA; 1.0 μ g/mL) solution in pH 7.5 dilution buffer made of 0.1 M NaPO₄, 0.8 M KF, and 0.1% BSA. After the mixture had been incubating at room temperature for 15 min, 100 μ L (per well) of the reaction mixture was transferred to a 96-well microplate (Corning #3693 96-well, white polystyrene, half area) in triplicates and HTRF emissions at 616 and 665 nm were measured by exciting at 340 nm using SpectraMax i3x Microplate Reader (Molecular Devices).

Heparanase Hydrolysis Assay⁴⁹

Micro centrifuge tubes were pretreated for 2 h for 37 °C by using a solution of phosphate buffered saline comprising of 0.05% Tween 20 (PBST) and 4% bovine serum albumin. Tubes were then washed three times with PBST, dried, stored at 4 °C, and used for assay within a week. The enzymatic reaction was stopped at different time points by adding equal volumes of reaction mixture and freshly made 1.69 mM WST-1 (Toronto Research Chemicals) to a new microtube containing 0.1 M NaOH solution. The resulting solution was developed at 60 °C for 60 min, and 200 μ L (per well) of developed reaction solution was transferred to a clear 96-well microplate in triplicate and absorbance was measured at 584 nm with a SpectraMax Plus 384 Microplate Reader (Molecular Devices). For the controls, exactly the same procedure was followed for a 100 μ M solution of polymer or Fondaparinux without heparanase enzyme to rationalize nonenzymatic autohydrolysis.

Computational Docking

For the docking studies, we utilized the *apo* heparanase structure (PDB code: 5E8M).³³ The enzyme structure was imported into Yasara,⁵⁰ cleaned, energy minimized in vacuo, and Glu225 was manually protonated. Ligands were constructed in a twostep method. The saccharide portion was first built using the Glycam GAGs builder (www.glycam.com) and then imported into the Avogadro molecular editing software (<https://avogadro.cc/>), where aliphatic portion was added. The ligand was then subjected to a steepest descent energy minimization and saved in the .pdb format. Global docking with each ligand was performed on the heparanase structure separately using the Autodock VINA⁵¹ default parameters in a simulation cell set built at least 10 Å from all the three sides of the enzyme. The setup was done with the YASARA molecular modeling program (www.yasara.org) and the built-in docking simulation macro “dock_run.mcr” for 100 docking runs using the AMBER14 force field for protein⁵² and GLYCAM06⁵³ and GAFF/Am1BCC for the synthetic saccharide ligand and a pose cluster RMSD of 5 Å for the docking conformations. Ligands and receptor residues were kept flexible during the docking runs. The most populated clusters of the 100 docking runs was subjected to further analysis.

Molecular Dynamic Simulations

All the simulations were performed in the Yasara Structure, version 16.4.6,⁵⁰ with the built-in molecular dynamic simulation macro “md_run.mrc”. The heparanase–ligand complexes

were imported into Yasara and energy minimized in vacuo. Subsequently, a simulation cell was built with at least 10 Å from all the three sides of the enzyme–ligand complex molecule. AMBER14 force field was utilized with periodic boundary conditions. The force field parameters were done YASARA's "AutoSMILES" feature and assigned the AMBER14 force field⁵² for the amino acid residues and GLYCAM06⁵³ oligosaccharide in conjunction with GAFF/AM1BCC for the atoms that were not part of GLYCAM. The simulation box was filled with TIP3P⁵⁴ water as solvent. All simulated systems were neutralized at pH 7.4 by counterions using 0.9% NaCl (concentration in mass percent, a physiological solution). Solvent density was 0.997 g/mL (water at 300 K). The pK_a of titrable side chains was predicted and assigned to respective groups before running MD and Glu225 was manually protonated.

Following the minimization of the contents of the simulation box, a molecular dynamics simulation was performed for 25 ns. The MD simulations were run at 298 K with Berendsen thermostat⁵⁵ and the pressure coupling was with manometer method as available in Yasara. The cutoff radius for long-range electrostatics was set to 8 Å. The snapshots were saved at 25 ps intervals for the length of the 25 ns simulation. The equations of motion were integrated with a 1.25 fs time step. To gain performance, the nonbonded van der Waals and electrostatic forces were evaluated only every second step, and added with a scaling factor of 2. This straightforward multiple time step algorithm is known as the "impulse method"⁵⁶ or "VERLET-I".⁵⁷ The pair list was updated every 10 steps ($10 \times 2.5 \text{ fs} = 25 \text{ fs}$). Snapshots were then analyzed using the built-in "md_analyze.mcr" macro for the RMSD of the $C\alpha$ of the α -helices and β -strands and the ligand heavy atoms as distances of the catalytic residues from the scissile bond of the ligand.

RESULTS AND DISCUSSION

Design and Computational Study of Monomers

The "glycocode" for heparanase recognition has been extensively investigated, and it has been established that most effective pattern for cleavage is represented by tetrasaccharide **4** (Figure 2, scissile bond shown in red), consisting of a repeating GlcNS(6S)-GlcA moiety.³⁴ From this pattern, it was chosen that the GlcNS(6S) α (1,4)GlcA unit of the $-2/-1$ subsites would be reflected in the pendant saccharide moiety (for glycosyl hydrolase nomenclature, see ref 58). We hypothesize that dismissal of the cleavable GlcA β (1,4)GlcNS(6S) moiety increases the hydrolytic stability of the molecule. In turn, use of the high affinity GlcNS(6S) α (1,4)GlcA unit of choice will potentially inhibit heparanase as it contains no scissile bond. Clustering of inhibitory moieties (inhibitopes) on multivalent scaffolds has been shown to inhibit glycosidases with high potency.^{19–30} Use of the GlcNS(6S) α (1,4)GlcA disaccharide is also advantageous, as it does not contain an iduronic acid unit nor a C(3)-sulfated glucosamine, diminishing cross-bioactivity.^{43,59–62} Furthermore, we hypothesize that attachment of this disaccharide to the aglycon polymerizable linker, which disrupts the hydrolytic ability of heparanase and provides π -cation interactions if an aromatic system is employed.^{63–65} However, this disaccharide moiety does contain the synthetically challenging α (1,4) glycosidic linkage.⁶⁶

Based on previous work by our group⁶⁷ and others,^{68–70} we hypothesize that use of a multiantennary monomer could increase the local saccharide density and have a greater avidity than the monoantennary counterpart. In that respect, we designed two different monomeric units (**5** and **6**, Figure 2) based on tetrasaccharide **4** (heparanase substrate) as HS mimics. The first would be the commonly used monoantennary type scaffold **7** with a terminal alkyne for later conjugation of the GlcNS(6S) α (1,4)GlcA disaccharide unit by a copper(I)-catalyzed alkyne–azide cycloaddition (CuAAC) “click” reaction to form monoantennary monomer **5** (Figure 1), replicating the –1/–2 binding epitope of **4**. Alternatively, we have also employed the Grubbs and Johnson’s diantennary bottlebrush scaffold **8**, which has been used in drug-delivery systems and possesses orthogonally functionalizable end groups (alkyne and carboxylic acid) for conjugating to two different saccharide units.^{67,71,72} Similar to that of the monoantennary, the GlcNS(6S) α (1,4)GlcA disaccharide could be attached via a CuAAC “click” reaction to diantennary scaffold **8**. A subsequent amide coupling by an additional GlcNS(6S) unit bearing a terminal amine linker would then form diantennary monomer **6** (Figure 2), which increases the local saccharide density to better mimic the heterogeneity of HS chains.^{4,73} Furthermore, we hypothesize that placement of a secondary sugar moiety in such close proximity enhances the probability of statistical rebinding and can also force a secondary unit into other binding sites, thus, allowing **6** to bind to both heparin binding domains (HBD-1 and HBD-2).^{20,23,6770} Additionally, the diantennary unit potentially mimics the trisaccharide that binds into the –1/–2/+1 subsites of heparanase, but removes the scissile bond, thereby preventing cleavage by bridging over the catalytic residues.^{31,32} Knowing the desire for a homogeneous polymer with uniform distribution of the pendant saccharides, a grafting-through method of polymerization was to be used.⁷²

In order to approximate how these ligands would bind to human heparanase and maintain the interactions of the natural substrate, diantennary monomer **6** (Figure 3A) and monoantennary monomer **5** (Figure 3B) were then constructed in silico to be docked in the apo heparanase crystal structure (PDB code: 5E8M)³³ employing the Autodock Vina portion of the YASARA Structure Program.^{49,50} It is noted that due to their structural complexity, at this time there is no computational program that can readily and precisely manage the docking of the corresponding neo-glycopolymers of **5** and **6**. Upon examination of the initial docked structures for the monomer–heparanase complexes, both monomers **5** and **6** placed the GlcNS(6S) α (1,4)GlcA into subsites –1/–2 (Figure 3) in a similar fashion to Davies’ cocrystallized HS-heparanase structures (Figure 2).³³ The disaccharide was oriented in the same direction as the cocrystallized structures with the nonreducing end toward HBD-1 and the reducing end closer to HBD-2. When looking at specific interactions for both glycomonomers, the *N*-sulfate of the –2 subsite glucosamine, formed a strong trivalent hydrogen bonding network with Asn64, Gly389, and Tyr391 (Figure 3C,E). While –2 C(6) sulfate of diantennary monomer **6** had formed a salt bridge and hydrogen-bonded to Lys159 (Figure 3C), monoantennary monomer **5**’s –2 glucosamine C(6) sulfate had formed a hydrogen bond to Arg303 (Figure 3E). The attached aliphatic scaffold was found in the binding groove forming a network of hydrophobic, π -cation, and hydrogen bonding interactions.^{63–65} Intriguingly, the triazole formed by “click” reaction provided several of these π -cation and hydrogen bonding interactions with the heparanase active site.⁶³ On the

other hand, the strained bicyclic ring of the scaffold tended to stay above the binding groove. The major difference between these two monomers came from additional GlcNS(6S) in the diantennary **6**, which is able to interact with HBD-2 by forming several hydrogen bonds as well as an ionic salt bridge between the C(6) sulfate and Arg272 (Figure 3D), whereas the monoantennary **6** formed only minimal interactions with HBD- 2 (Figure 3B).

These ligand–enzyme complexes were subjected to a 25 ns MD simulation and established the complex's stability and the distance between the scissile bond of diantennary monomer **6** (Figure 4A) and monoantennary monomer **5** (Figure 4B) to the catalytic residues Glu225 and Glu343.^{74,75} As illustrated in Figure 4, the arrangement and distances of the cleavable bond to the catalytic residues, Glu225 and Glu343, for diantennary monomer **6**, is now farther and skewed away (4.53 and 5.04 Å) from the requisite *anti* alignment when compared to the natural tetrasaccharide substrate **4** (3.2 and 3.75 Å; see SI), making hydrolysis unlikely (Figure 4A).⁷⁴ On the other hand, monoantennary monomer **5** (Figure 4B) preferred an alignment and distance more similar to the natural substrate **4** by maintaining a close distance to the nucleophilic Glu343 (3.04 Å).

Synthesis of Monoantennary Monomer

With the docking results in hand, our next goal was to develop an efficient strategy for the synthesis of both monomers **5** (Scheme 1) and **6** (Scheme 2). To design a fully protected GlcNS α (1,4)GlcA moiety compatible with the ROMP event and the selective sulfation, we chose to use the naphthylmethyl (NAP) and the acetyl (Ac) protecting groups for both glucosamine-derived donor **9** and glucuronic acid acceptor **10** (Scheme 1). By employing our nickel-catalyzed 1,2-*cis*-2-aminoglycoside method,^{76–80} the glycosylation of protected glucuronic acid **10** possessing the azide linker with *N*-phenyl trifluoroacetimidate donor **9** was investigated in 15 mol % of Ni(OTf)₂ (Scheme 1). The reaction proceeded smoothly to provide an exclusive α -disaccharide **11**, a precursor of GlcNS α (1,4)GlcA, in 79% yield. The method overcomes the limitations previously associated with the stereoselective coupling of the electron-poor glucuronic acid acceptor with a glucosamine-derived donor.⁶⁶ Next, selective hydrolysis of the C(6)-acetyl group in disaccharide **11** was examined. Fortunately, subjection of **11** to NaOMe for 2 h at 25 °C produced the disaccharide-containing primary alcohol intermediate, whose *N*-benzylidene protecting group was then removed with HCl in acetone. Selective sulfation using sulfur trioxidetrimethyl amine (SO₃·Me₃N) yielded the corresponding sulfated disaccharide **12** (Scheme 1) in 65% over three steps. The NAP groups were removed with DDQ to yield **13** prior to the ROMP event. Subsequent CuAAC “click” reaction of azide **13** with the terminal alkyne of monoantennary scaffold **7** provided **14** in 64% yield, which will be used in the later ROMP event to generate glycopolymers (Table 1). Hydrolysis of both the C(3)-acetyl group and methyl ester of **14** completed the synthesis of monoantennary monomer **5** (Scheme 1).

Synthesis of Diantennary Monomer

The synthesis of diantennary monomer **6** (Scheme 2) with the additional GlcNS unit required the nickel-catalyzed glycosylation of ethylene glycol-containing azide acceptor **16** with glucosamine-derived donor **15** to produce the 1,2-*cis*-aminoglycoside **17** in 75% yield (α -anomer).^{76–80} Removal of both C(6)-acetyl and C(2)-*N*-benzylidene groups followed by

selective sulfation furnished disulfated monosaccharide **18** (Scheme 2) in 73% over three steps. Utilizing palladium hydroxide on carbon, Pd(OH)₂/C, under a H₂ atmosphere, simultaneously removed the C(4)-benzyl ether and reduced the terminal azide of **18** to the corresponding amine-containing product **19** in 96% yield. Subsequent amide coupling of amine **19** with *N*-hydroxysuccinimide ester **20** afforded the monosaccharide-linked polymerizable intermediate **21**, which then underwent a “click” reaction with disaccharide **12** (Scheme 1) to provide the protected monomer **22** in 92% yield. The 2-naphthylmethyl ethers (NAP) were then removed with DDQ to yield monomer **23** in 86% yield. Both monomers **22** and **23** would be used for later polymerizations to examine the utility of the NAP protecting groups in the synthesis of glycopolymers. Finally, hydrolysis of both the methyl ester and the C(3)-acetyl generated diantennary monomer **6** in 98%.

Ring Opening Metathesis Polymerization (ROMP)

With monomers in hand, we then explored their polymerization to the corresponding multivalent neo-glycopolymers. There are two important criteria to keep in mind when polymerizing monomers to generate the corresponding HS neo-glycopolymers. The first criteria is degrees of polymerization (DP). Many studies have shown that there is a direct correlation between the length of glycopolymers and how strong the binding is.^{41,81,82} Use of the strained oxanorborene ring motif in the ROMP system have been known to allow for highly controllable DP by varying the catalytic loading.⁸³ The next criteria is the solvent system of choice. Traditional ROMP solvents such as methylene chloride are not able to solvate the sulfated monomer. Use of an aqueous system requires the synthesis of novel water-soluble ruthenium catalysts, and so there exists a careful balance in solvent choice.⁸⁴ To overcome the polarity issue, we hypothesize that the 2-naphthylmethyl groups could increase the solubility in nonpolar solvents.⁴³ In our preliminary studies, the polymerization of the NAP-protected diantennary monomer **22** with third generation Grubbs catalyst **24** proceed smoothly. Unfortunately, thorough screening of hydrogenation conditions to remove NAP ethers only resulted in partial deprotection or, if exposed to harsher conditions (1000 psi H₂, 60 °C, 4 d), resulted in decomposition. In turn, it was chosen to deprotect the NAP groups with DDQ (see **14** and **23**, Schemes 1 and 2) prior to polymerization, making for less manipulations after polymerization. The solvent polarity issue was then addressed by utilization of weakly coordinating polar 2,2,2-trifluoroethanol (TFE) as part of the cosolvent along with 1,2-dichloroethane (DCE) allowing for an increased ratio without lowering efficacy of the catalyst (Table 1 and Scheme 3).⁸⁵

Accordingly, ROMP of monoantennary monomer **14** was investigated with Grubbs third generation catalyst **24** (Table 1).⁸³ By using 2.5:1 ratio of DCE/TFE, the polymerization reached complete conversion (100%) in less than 1 h (entries 1 and 2). Tuning of the amount of catalyst **24** (10 and 30 mol %) allowed for precise and repeatable control over the length of the glycopolymers **25** (DP = 5) and **26** (DP = 9), respectively, in high yield (entries 1 and 2). With a desire to achieve increased DP, the catalytic loading was decreased to 7 mol % and solvent ratio changed to 1:1 DCE/TFE (entry 3). Use of these new conditions allowed for polymerization of the monoantennary polymer **27** with 18 repeating units (DP = 18), although with a slight diminished in conversion to 81% (entry 3). To obtain pure polymer **27** (DP = 18), tedious removal of the monomer **14** was accomplished by repetitive

precipitations from methanol by a 5:1 DCE/TFE mixture resulting in the reduced isolated yield (40%). Due to an increase in amphiphilic nature of the sulfated saccharide and polymer backbone, analysis of glycopolymers **25–27** by SEC/MALLS only resulted in characterization of the micellular form. Screening of a wide variety of solvents and salt concentrations could not dissipate the micelle or in other solvents the polymer was insoluble. As a result, these glycopolymers could not be analyzed by GPC. Therefore, degrees of polymerization (DP) of **25–27** were determined by ^1H NMR end group analysis.⁸⁶ These polymers were then hydrolyzed using LiOH in an aqueous/THF mixture, followed by dialysis against a 0.9% saline solution, to provide the corresponding monoantennary glycopolymers **28–30** (Table 1), for biological studies of their inhibitory activities against heparanase.

For the ROMP of diantennary monomer **23** (Scheme 3), the ratio of DCE/TFE to 1:1 was the most efficient cosolvent for polymerization with 10 mol % of Grubbs third generation catalyst **24**. The reaction was complete in less than 1 h, and the corresponding glycopolymer **31** (DP = 8) was isolated in 81% yield. Hydrolysis of the C(3)-acetyl and methyl ester groups with LiOH provided diantennary polymer **32** (Scheme 3). Unfortunately, use of 7 mol % Grubbs third generation catalyst **24** with diantennary monomer **23** to increase molecular weight (M_n) of glycopolymers only resulted in low conversion and premature precipitation. Nevertheless, we were able to achieve equal DP for both types of monomers **14** (Table 1) and **23** (Scheme 3) to have a direct comparison for the inhibition of heparanase.

Heparanase Inhibition

The new glycopolymers were then screened for their inhibition of heparanase using an in vitro TR-FRET assay against fluorescence-labeled heparan sulfate to test the effect of increased carbohydrate density and better mimicry of HS chains (diantennary vs monoantennary)⁴⁸ along with control glucosamine monosaccharide **33** and disaccharide **34**, as well as oligosaccharides **35** and **36** (Figure 5).^{87–90} Glucosamine **33** bearing the sulfate group at C(6)-*O* and C(2)-*N* positions will be used to determine whether the glucuronic acid (GlcA) is important for inhibition against heparanase. Disaccharide **34** will be used to determine whether the polymerizable scaffold embedded in the designed monomers **5** and **6** plays a role in binding affinity. Use of oligosaccharides **34** and **35** will determine the importance of multivalency of glycopolymers **28–30** and **32** in the binding event.

Initial studies commenced with subsection of the monomer units **5** and **6** to the TR-FRET inhibition assay (Figure 6). As expected, diantennary monomer **6** with the additional GlcN-(6S) appendage ($\text{IC}_{50} = 31.23 \pm 2.36 \mu\text{M}$, entry 4) is more potent than monoantennary monomer **5** ($\text{IC}_{50} = 49.44 \pm 1.56 \mu\text{M}$, entry 3). These IC_{50} values are comparable to those of previous heparanase small molecule inhibitors.³¹ The difference in binding between **5** and **6** could be due to **6** having little interaction with HBD-2. It should be noted that **5** exhibited no increase in binding when compared to that of control disaccharide **34** with no attached scaffold ($\text{IC}_{50} = 50.07 \pm 3.46 \mu\text{M}$, entry 2). The results suggest that the strained oxanorbornene ring system had no influence on binding and are consistent with in the MD simulation (Figure S2, the strain ring system had no interactions with the enzyme). The low inhibitory activity of the control disulfated glucosamine derivative **33** ($\text{IC}_{50} = 464.4 \pm 20.8$

μM , entry 1) not only exemplifies the additional potency of using a disaccharide over a monosaccharide unit, but also conveys the importance of the glucuronic acid unit as part of the glycotope for heparanase recognition.

Having confirmed the ability of monomers **5** and **6** (Figure 6, entries 3 and 4) to act as heparanase inhibitors, we next questioned whether their corresponding neo-glycopolymers could exhibit higher activity. It was determined that, even at short glycopolymer **28** (DP = 5), the newly formed multivalent structure amplified the affinity to heparanase by over 1000-fold ($\text{IC}_{50} = 15.48 \pm 1.6 \text{ nM}$, entry 7). Only the best HS mimicking carbohydrate-based inhibitors have been able to inhibit in the nanomolar range.³¹ The results obtained with the use of both octasaccharide **35** ($\text{IC}_{50} = 3.94 \pm 0.20 \mu\text{M}$, entry 5) and heptasaccharide **36** ($\text{IC}_{50} = 41.68 \pm 3.64 \mu\text{M}$, entry 6) as control substrates further demonstrate the power of multivalent compounds in effecting the binding. Although oligosaccharides **35** and **36** are slightly smaller than monoantennary glycopolymer **28**,^{87–90} they are significantly less potent than **28**. Furthermore, the increase in the degrees of polymerization (DP) had a direct effect on the binding affinity, which has been reported in many previous neo-glycopolymer binding studies.^{41,81} For instance, increasing DP of the monoantennary polymer **29** to 9 repeating units had a direct effect on the binding affinity (DP = 9, $\text{IC}_{50} = 6.63 \pm 0.27 \text{ nM}$, entry 8), which is more potent than polymer **28** with a smaller degree of polymerization (DP = 5, entry 7). Further increase of the degrees of polymerization results in higher binding affinity as the glycopolymer **30** with 18 repeating units was determined to be the most potent heparanase inhibitor with $\text{IC}_{50} = 1.05 \pm 0.02 \text{ nM}$ (entry 9). The polymer **30** (DP = 18) is almost as potent as unfractionated heparin ($\text{IC}_{50} = 0.54 \pm 0.028 \text{ nM}$, entry 11); however, the sulfation pattern is unknown for heparin. The powerful utility of multivalency was truly observed in the affinity enhancement on a disaccharide molar basis (Figure 6). Monoantennary polymer **30** (DP = 18) had an enhancement of nearly 2600-fold, whereas diantennary polymer **32** (DP = 8) had an enhancement of 1123-fold over their monovalent counterparts.

In a direct inhibition comparison of two different saccharides attached to the backbone of polymers, monoantennary polymer **29** (DP = 9, $\text{IC}_{50} = 6.63 \pm 0.27 \text{ nM}$, entry 8) had less binding affinity than diantennary polymer **32** (DP = 8, $\text{IC}_{50} = 5.49 \pm 0.18 \text{ nM}$, entry 10). Although still highly potent, diantennary polymer **32** (DP = 8) is not as amplified as much as expected, which could be the result of a steric clash of the additional GlcNS(6S) moiety in the deep binding groove along with the electronic repulsion of the two negatively charged appendages. In the docking studies, another ligand–heparanase complex cluster with a slightly lower population oriented the monosaccharide unit above the disaccharide unit (Figure S2). Although the steric repulsion had little influence during the monomer inhibition experiment, the effect could be intensified once the glycomonomer is polymerized. It also seems the overarching multivalent presentation of saccharides dwarfed that of the specific presentation for this heparanase application; however, it has potential to be applicable to other HS binding proteins. The small increase in affinity could also be due to having linkers that were too long and did not appropriately match the distance of the binding domains inducing a high enthalpic cost to place both units into the binding site. Studies to modify these lengths are current underway to determine its effect on affinity.

Heparanase Hydrolysis

Since the inhitope motif was a derivative of the natural substrate of heparanase, there is potential for the possibility of hydrolysis of the saccharide unit from the polymeric backbone. To confirm that use of an aglycon linker prevented this occurrence, a colorimetric assay was performed using a tetrazolium sodium salt (WST-1), which reacts with a newly formed anomeric hemiacetal.⁴⁹ Monitoring the degradation over a period of 23 h showed a drastic change in absorbance for the ultralow molecular weight heparin substrate, Fondaparinux, by over 2-fold (Figure 7). Fondaparinux is known to be cleaved by heparanase. In contrast, use of diantennary polymer **32** (DP = 8) and monoantennary polymer **29** (DP = 9) in this type of experiment had only a slight change in absorbance over the same amount of time. To determine if autohydrolysis occurred during the assay due to use of acidic media, each experiment were run concurrently with a control of polymer without enzyme. From these control studies, no acid-catalyzed hydrolysis occurred and any change in absorbance was due to glycosidic cleavage by heparanase. For the monoantennary substrate **29**, there was a small increase at the 10 h time point, which could be attributed to the close distance of the scissile bond to the catalytic residues seen during the MD simulations of its monovalent unit (Figure 4B), thus, allowing for cleavage to be possible.⁷⁴ Overall, this cleavage is minute in comparison to that of Fondaparinux, which itself is a poor substrate for heparanase.

Physiochemical Characterization

Furthermore, it is well-known that an amphiphilic bottlebrush monomer configuration with a drastic difference in polarity such as glycomonomers **5** and **6** (hydrophobic = polymer backbone, hydrophilic = sulfated saccharides) are prone to self-aggregate into micelles spontaneously after polymerization.⁸ After ROMP and global deprotection, the resulting neo-glycopolymers **28–30** (Table 1) and **32** (Scheme 3) spontaneously formed micelles very efficiently. Using dynamic light scattering (DLS), these micelles had *Z*-average hydrodynamic radii's between 60 and 70 nm and low polydispersities (PDI = 19.1–22.6%; Table 2 and Figure S3), suggesting these micelles have high degrees of homogeneity. Next, negative staining by 1% uranyl acetate of both the diantennary polymer **32** (DP = 8) and monoantennary polymer **29** (DP = 9) confirmed with transmission electron microscopy (TEM) that regardless of monomer configuration both of them assembled into spherical micelles (Figure 8). However, the micelles formed by diantennary polymer **32** (Figure 8B) appeared to be more isolated, whereas the micelles from monoantennary polymer **29** were typically found in closer proximity (Figure 8A). Further analysis underway to determine if the clustering is a property of the monoantennary polymer or an artifact of sample preparation.

Lastly, the critical micelle concentration (CMC) was determined for both the diantennary polymer **32** (DP = 8) and monoantennary polymer **29** (DP = 9), separately, by using pyrene as a fluorogenic probe.⁴⁷ By comparing the ratio of the first (373 nm) and third (384 nm) vibronic peaks of emission (I_1/I_3), while varying the concentrations of each polymer, the CMC was determined to be at the downward break of this ratio where pyrene began to be entrapped in the nonpolar environment of the micelle interior (monoantennary = 8.5 μ M, diantennary = 4 μ M, Figure 9). We hypothesize that the additional sulfates on the

diantennary polymer **32** increased the overall amphiphilicity, thus, lowering the CMC. To this end, since the CMC is near 1000-fold greater than the IC₅₀ value, we hypothesize that the inhibition of heparanase is predominately exerted by the unicellular linear neo-glycopolymers. The mode of binding for these neo-glycopolymers is under further investigation and will be reported in due time.

CONCLUSION

We described the rational design and proper choice of sulfation pattern for, to the best of our knowledge, the unprecedented use in the development of synthetic glycopolymers for the inhibition of glycosidase. As a proof of concept, the glycopolymers were designed by methodical reading of the “glycocode” of the particular cancer-linked GAGs degrading enzyme, heparanase, and placement of the properly sulfated saccharides pendants onto a polymer scaffold. In turn, the glycopolymers with the pendant inhitope were capable of inhibiting heparanase in low nanomolar concentrations, which are 1000-fold more potent than their monovalent counterparts. Although the exact mode of inhibition of these potent synthetic glycopolymers as glycosidase inhibitors is presently being investigated, these results emphasize the power of multivalent inhibition on carbohydrate-degrading enzymes. Importantly, it was determined that the incorporation of an additional monosaccharide unit can play a unfavorable role when inhibiting heparanase due to its deep active sites, thus, limiting the amount of substrate capable of fitting into the pocket for inhibition due to steric interactions. Overall, these results provide the platform that could be applicable to not only HS-degrading enzymes but to a wide variety of carbohydrate degrading enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Dwek RA. Chem Rev. 1996; 96:683–720. [PubMed: 11848770]
2. Kiessling LL, Grim JC. Chem Soc Rev. 2013; 42:4476–4491. [PubMed: 23595539]
3. Dam TK, Brewer CF. Chem Rev. 2002; 102:387–429. [PubMed: 11841248]
4. Lundquist JJ, Toone EJ. Chem Rev. 2002; 102:555–578. [PubMed: 11841254]
5. Miura Y. Polym J. 2012; 44:679–689.
6. Mammen M, Choi SK, Whitesides GM. Angew Chem, Int Ed. 1998; 37:2754–2794.
7. Miura Y, Hoshino Y, Seto H. Chem Rev. 2016; 116:1673–1692. [PubMed: 26509280]

8. Ladmiraal V, Melia E, Haddleton DM. Eur Polym J. 2004; 40:431–449.
9. Gestwicki JE, Cairo CW, Strong LE, Carolyn AO, Kiessling LL. J Am Chem Soc. 2002; 124:14922–14933. [PubMed: 12475334]
10. Spaltenstein A, Whitesides GM. J Am Chem Soc. 1991; 113:686–687.
11. Cloninger MJ. Curr Opin Chem Biol. 2002; 6:742–748. [PubMed: 12470726]
12. Martinez A, Ortiz Mellet C, Garcia Fernandez JM. Chem Soc Rev. 2013; 42:4746–4773. [PubMed: 23340678]
13. Sansone F, Casnati A. Chem Soc Rev. 2013; 42:4623–4639. [PubMed: 23361847]
14. Koide H, Yoshimatsu K, Hoshino Y, Lee SH, Okajima A, Ariizumi S, Narita Y, Yonamine Y, Weisman AC, Nishimura Y, Oku N, Miura Y, Shea KJ. Nat Chem. 2017; 9:715. [PubMed: 28644480]
15. Lundquist JJ, Debenham SD, Toone EJ. J Org Chem. 2000; 65:8245–8250. [PubMed: 11101380]
16. Kikkeri R, Lepenies B, Adibekian A, Laurino P, Seeberger PH. J Am Chem Soc. 2009; 131:2110–2112. [PubMed: 19199612]
17. Mortell KH, Gingras M, Kiessling LL. J Am Chem Soc. 1994; 116:12053–12054.
18. Fraser C, Grubbs RH. Macromolecules. 1995; 28:7248–7255.
19. Gouin SB. Chem - Eur J. 2014; 20:11616–11628. [PubMed: 25081380]
20. Garcia-Moreno MI, Ortega-Caballero F, Ríquez-Cuadro R, Mellet CO, Fernandez JMG. Chem - Eur J. 2017; 23:6295–6304. [PubMed: 28240441]
21. Compain P, Decroocq C, Iehl J, Holler M, Hazelard D, Mena Barragan T, Ortiz Mellet C, Nierengarten J-F. Angew Chem, Int Ed. 2010; 49:5753–5892.
22. Abellan Flos M, Garcia Moreno MI, Ortiz Mellet C, Garcia Fernandez JM, Nierengarten J-F, Vincent SP. Chem - Eur J. 2016; 22:11450–11460. [PubMed: 27374430]
23. Ortiz Mellet C, Nierengarten J-F, Garcia Fernandez JM. J Mater Chem B. 2017; 5:6428–6436.
24. Bonduelle C, Huang J, Mena-Barragan T, Ortiz Mellet C, Decroocq C, Etame E, Heise A, Compain P, Lecommandoux S. Chem Commun. 2014; 50:3350–3352.
25. Decroocq C, Rodriguez-Lucena D, Russo V, Mena-Barragan T, Ortiz Mellet C, Compain P. Chem - Eur J. 2011; 17:13825–13831. [PubMed: 22052823]
26. Decroocq C, Joosten A, Sergent R, Mena Barragan T, Ortiz Mellet C, Compain P. ChemBioChem. 2013; 14:2038–2049. [PubMed: 24014313]
27. Joosten A, Schneider JP, Lepage ML, Tarnus C, Bodlenner A, Compain P. Eur J Org Chem. 2014; 2014:1866.
28. Alvarez-Dorta D, King DT, Legigan T, Ide D, Adachi I, Deniaud D, Désiré J, Kato A, Vocadlo D, Gouin SG, Blériot Y. Chem - Eur J. 2017; 23:9022–9025. [PubMed: 28548311]
29. Siriwardena A, Khanal M, Barras A, Bande O, Mena-Barragan T, Ortiz Mellet C, Garcia Fernandez JM, Boukherroub R, Szunerits S. RSC Adv. 2015; 5:100568–100578.
30. Brissonnet Y, Ladeveze S, Teze D, Fabre E, Deniaud D, Daligault F, Tellier C, Sestak S, Remaud-Simeon M, Potocki-Veronese G, Gouin SG. Bioconjugate Chem. 2015; 26:766–772.
31. Rivara S, Milazzo FM, Giannini G. Future Med Chem. 2016; 8:647–680. [PubMed: 27057774]
32. Vlodavsky I, Singh P, Boyango I, Gutter-Kapon L, Elkin M, Sanderson RD, Ilan N. Drug Resist Updates. 2016; 29:54–75.
33. Wu L, Viola CM, Brzozowski AM, Davies GJ. Nat Struct Mol Biol. 2015; 22:1016–1022. [PubMed: 26575439]
34. Peterson SB, Liu J. Matrix Biol. 2013; 32:223–227. [PubMed: 23499529]
35. Ziolkowski AF, Popp SK, Freeman C, Parish CR, Simeonovic CJ. J Clin Invest. 2012; 122:132–141. [PubMed: 22182841]
36. van den Hoven MJ, Rops AL, Vlodavsky I, Levidiotis V, Berden JH, van der Vlag J. Kidney Int. 2007; 72:543–548. [PubMed: 17519955]
37. Rabelink TJ, van den Berg BM, Garsen M, Wang G, Elkin M, van der Vlag J. Nat Rev Nephrol. 2017; 13:201–212. [PubMed: 28163306]
38. Grande D, Baskaran S, Baskaran C, Gnanou Y, Chaikof EL. Macromolecules. 2000; 33:1123–1125.

39. Grande D, Baskaran S, Chaikof EL. *Macromolecules*. 2001; 34:1640–1646.
40. Baskaran S, Grande D, Sun XL, Yayon A, Chaikof EL. *Bioconjugate Chem*. 2002; 13:1309–1313.
41. Sun XL, Grande D, Baskaran S, Hanson SR, Chaikof EL. *Biomacromolecules*. 2002; 3:1065–1070. [PubMed: 12217054]
42. Qian Y, Pan J, Zhou X, Weiser P, Lu H, Zhang L. *Prog Mol Biol Transl Sci*. 2010; 93:395–421. [PubMed: 20807654]
43. Oh YI, Sheng GJ, Chang SK, Hsieh-Wilson LC. *Angew Chem, Int Ed*. 2013; 52:11796–11799.
44. Sheng GJ, Oh YI, Chang SK, Hsieh-Wilson LC. *J Am Chem Soc*. 2013; 135:10898–10901. [PubMed: 23879859]
45. Miura Y, Fukuda T, Seto H, Hoshino Y. *Polym J*. 2016; 48:229–237.
46. Sarrazin S, Lamanna WC, Esko JD. *Cold Spring Harbor Perspect Biol*. 2011; 3:a004952.
47. Kalyanasundaram K, Thomas JK. *J Am Chem Soc*. 1977; 99:2039–2044.
48. Roy S, El Hadri A, Richard S, Denis F, Holte K, Duffner J, Yu F, Galcheva-Gargova Z, Capila I, Schultes B, Petitou M, Kaundiny GV. *J Med Chem*. 2014; 57:4511–4520. [PubMed: 24786387]
49. Hammond E, Li CP, Ferro V. *Anal Biochem*. 2010; 396:112–116. [PubMed: 19748475]
50. Krieger E, Vriend G. *Bioinformatics*. 2014; 30:2981–2982. [PubMed: 24996895]
51. Trott O, Olson AJ. *J Comput Chem*. 2010; 31:455–461. [PubMed: 19499576]
52. Case DA, , Babin V, , Berryman JT, , Betz RM, , Cai Q, , Cerutti DS, , Cheatham TE, , III, Darden TA, , Duke RE, , Gohlke H, , Goetz AW, , Gusarov S, , Homeyer N, , Janowski P, , Kaus J, , Kolossváry I, , Kovalenko A, , Lee TS, , LeGrand S, , Luchko T, , Luo R, , Madej B, , Merz KM, , Paesani F, , Roe DR, , Roitberg A, , Sagui C, , Salomon-Ferrer R, , Seabra G, , Simmerling CL, , Smith W, , Swails J, , Walker RC, , Wang J, , Wolf RM, , Wu X, , Kollman PA. AMBER 14 University of California; San Francisco: 2014
53. Kirschner KN, Yongye AB, Tschampel SM, Gonzalez-Outeirino JG, Daniels CR, Foley BL, Woods RJ. *J Comput Chem*. 2008; 29:622–655. [PubMed: 17849372]
54. Mahoney MW, Jorgensen WL. *J Chem Phys*. 2000; 112:8910–8914.
55. Berendsen HJC, Postma JPC, van Gunsteren WF, DiNola A, Haak JR. *J Chem Phys*. 1984; 81:3684–3690.
56. Grübmüller H, Tavan P. *J Comput Chem*. 1998; 19:1534–1552.
57. Grübmüller H, Heller H, Windemuth A, Schulten K. *Mol Simul*. 1991; 6:121–142.
58. Davies GJ, Wilson KS, Henrissat B. *Biochem J*. 1997; 321:557–559. [PubMed: 9020895]
59. Petitou M, van Boeckel CAA. *Angew Chem, Int Ed*. 2004; 43:3118–3133.
60. Pellegrini L, Burke DF, von Delft F, Mulloy B, Blundell TL. *Nature*. 2000; 407:1029–1034. [PubMed: 11069186]
61. Turnbull JE, Fernig DE, Kie Y, Wilkinson MC, Gallagher JT. *J Biol Chem*. 1992; 267:10337–10341. [PubMed: 1587820]
62. Stringer SW, Gallagher JT. *J Biol Chem*. 1997; 272:20508–20514. [PubMed: 9252363]
63. Sigal GB, Mammen M, Dahmann G, Whitesides GM. *J Am Chem Soc*. 1996; 118:3789–3800.
64. Ferro V, Liu L, Johnstone KD, Wimmer N, Karoli T, Handley P, Rowley J, Dredge K, Li CP, Hammond E, Davis K, Sarimaa L, Harenberg J, Bytheway I. *J Med Chem*. 2012; 55:3804–3813. [PubMed: 22458531]
65. Pearson AG, Kiefel MJ, Ferro V, von Itzstein M. *Org Biomol Chem*. 2011; 9:4614–4625. [PubMed: 21505696]
66. Dulaney SB, Huang X. *Adv Carbohydr Chem Biochem*. 2012; 67:95–136. [PubMed: 22794183]
67. Loka RS, McConnell MS, Nguyen HM. *Biomacromolecules*. 2015; 16:4013–4021. [PubMed: 26580410]
68. David A, Kopecková P, Rubinstein A, Kopeček J. *Bioconjugate Chem*. 2001; 12:890–899.
69. Sanders WJ, Gordon EJ, Dwir O, Beck PJ, Alon R, Kiessling LL. *J Biol Chem*. 1999; 274:5271–5278. [PubMed: 10026133]
70. Pieters RJ. *Org Biomol Chem*. 2009; 7:2013–2025. [PubMed: 19421435]
71. Johnson JA, Lu YY, Burts AO, Lim YH, Finn MG, Koberstein JT, Turro NJ, Tirrell DA, Grubbs RH. *J Am Chem Soc*. 2011; 133:559–566. [PubMed: 21142161]

72. Johnson JA, Lu YY, Burts AO, Xia Y, Durrell AC, Tirrell DA, Grubbs RH. *Macromolecules*. 2010; 43:10326–10335. [PubMed: 21532937]
73. Blanco JLJ, Melleta CO, Fernandez JMG. *Chem Soc Rev*. 2013; 42:4518–4531. [PubMed: 22911174]
74. Gandhi NS, Freeman C, Parish CR, Mancera RL. *Glycobiology*. 2012; 22:35–55. [PubMed: 21746763]
75. Pala D, Rivara S, Mor M, Milazzo FM, Roscilli G, Pavoni E, Giannini G. *Glycobiology*. 2016; 26:640–654. [PubMed: 26762172]
76. Sletten ET, Ramadugu SK, Nguyen HM. *Carbohydr Res*. 2016; 435:195–207. [PubMed: 27816838]
77. Mensah EA, Nguyen HM. *J Am Chem Soc*. 2009; 131:8778–8780. [PubMed: 19496537]
78. Mensah EA, Yu F, Nguyen HM. *J Am Chem Soc*. 2010; 132:14288–14302. [PubMed: 20860359]
79. Yu F, Nguyen HM. *J Org Chem*. 2012; 77:7330–7343. [PubMed: 22838405]
80. McConnell MS. PhD Dissertation University of Iowa; Iowa City, IA: 2015 Nickel catalyzed formation of 1,2-cis-2-amino sugars to access important biomolecules.
81. Strong LE, Kiessling LL. *J Am Chem Soc*. 1999; 121:6193–6196.
82. Fox ME, Szoka FC, Fréchet JMJ. *Acc Chem Res*. 2009; 42:1141–1151. [PubMed: 19555070]
83. Choi TL, Grubbs RH. *Angew Chem, Int Ed*. 2003; 42:1743–1746.
84. Leitgeb A, Wappel J, Slugovc C. *Polymer*. 2010; 51:2927–2946.
85. Rankin DA, P'Pool SJ, Schanz HJ, Lowe AB. *J Polym Sci, Part A: Polym Chem*. 2007; 45:2113–2128.
86. Radzinski SC, Foster JC, Chapleski RC, Troya D, Matson JB. *J Am Chem Soc*. 2016; 138:6998–7004. [PubMed: 27219866]
87. Liu C, Sheng J, Krahn JM, Perera L, Xu Y, Hsieh PH, Dou W, Liu J, Pederson LC. *J Biol Chem*. 2014; 289:13407–13418. [PubMed: 24652287]
88. Wang Z, Hsieh P-H, Xu Y, Thieker D, Juan En Chai E, Shaushuai X, Cooley B, Woods RJ, Chi L, Liu J. *J Am Chem Soc*. 2017; 139:5249–5256.
89. Yang J, Hsieh PH, Liu X, Zhou W, Zhang X, Zhao J, Xu Y, Zhang F, Linhardt RJ, Liu J. *Chem Commun*. 2017; 53:1743–1746.
90. Peterson S, Liu J. *J Biol Chem*. 2012; 287:34836–34843. [PubMed: 22893710]

Figure 1.
Heparan sulfate (HS) polysaccharide **1** and the designed neoglycopolymers monoantennary **2** and diantennary **3**.

Figure 2.
Designed monoantennary and diantennary monomers.

Figure 3.

Structures docked into human heparanase: (A) diantennary monomer **6**, (B) monoantennary monomer **5**, (C) hydrogen bonding network of disaccharide of diantennary monomer **6** in heparanase subsites -1/-2, (D) hydrogen bonding monosaccharide of diantennary monomer **6** to HBD-2, and (E) hydrogen bonding network of disaccharide of monoantennary monomer **5** in heparanase subsites -1/-2. Hydrogen bonding shown in magenta. HBD-2 is on the left and HBD-1 is on the right.

Figure 4. Distance between the scissile bond of diantennary monomer **6** (A) and monoantennary monomer **5** (B) to Glu225 and Glu343 over the 25 ns MD simulation.

Figure 5.
Control monosaccharide, disaccharide, and oligosaccharides.

^a Affinity enhancement is calculated on a disaccharide molar basis

Figure 6.
Effect of multivalency on the binding affinity using TR-FRET assay.

Figure 7. Hydrolytic potential over time of heparanase on monoantennary and diantennary polymers and Fondaparinux.

Figure 8.
TEM micrograph of monoantennary polymer **29** (DP = 9, **A**) and diantennary polymer **32** (DP = 8, **B**).

Figure 9.
CMC of monoantennary polymer **29** (DP = 9) and diantennary polymer **32** (DP = 8).

Scheme 1.
Stereoselective Synthesis of Monoantennary Monomer (5) via Nickel Catalysis

Scheme 2.
Stereoselective Synthesis of Diantennary Monomer (6)

Scheme 3.

ROMP of Monomer (23) to Generate Diantennary Glycopolymer

Table 1

ROMP of Monoantennary Monomer (14) with Third Generation Grubbs Catalyst (24)

entry	24 mol %	DCE/TFE	polymer	DP _n	conv (%)	yield (%)	M _n (theoretical)	M _n (NMR)	hydrolysis yield
1	30	2.5:1	25	5	100	97	3185	4865	28 (70%)
2	10	2.5:1	26	9	100	96	9730	8760	29 (75%)
3	7	1:1	27	18	81	40	13845	17520	30 (83%)

Table 2

Measuring the Size of Monodiantennary and Diantennary Glycopolymers Using DLS

entry	polymers	DP _n	R _h (nM)	PDI
1	32	8	71.0	20.1
2	28	5	59.2	20.0
3	29	9	68.2	19.1
4	30	18	64.7	22.6