

Probing Amyloid β Interactions with Synthetic Heparan Sulfate Oligosaccharides

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ABSTRACT: Heparan sulfate (HS) can play important roles in the biology and pathology of amyloid β ($A\beta$), a hallmark of Alzheimer's disease. To better understand the structure–activity relationship of HS/ $A\beta$ interactions, synthetic HS oligosaccharides ranging from tetrasaccharides to decasaccharides have been utilized to study $A\beta$ interactions. Surface plasmon resonance experiments showed that the highly sulfated HS tetrasaccharides bearing full 2-*O*, 6-*O*, and *N*-sulfations exhibited the strongest binding with $A\beta$ among the tetrasaccharides investigated. Elongating the glycan length to hexa- and deca-saccharides significantly enhanced $A\beta$ affinity compared to the corresponding HS tetrasaccharide. Solid state NMR studies of the complexes of $A\beta$ with HS hexa- and deca-saccharides showed most significant chemical shift perturbation in the C-terminus residues of $A\beta$. The strong binding HS oligosaccharides could reduce the cellular toxicities induced by $A\beta$. This study provides new insights into HS/ $A\beta$ interactions, highlighting how synthetic structurally well-defined HS oligosaccharides can assist in biological understanding of $A\beta$.

Alzheimer's disease (AD) is a neurodegenerative disease and the most common form of dementia, which has been estimated to afflict millions of people in the U.S. alone.¹ A prominent pathological hallmark of AD is amyloid- β ($A\beta$) plaques, where $A\beta$ polypeptides aggregate with nonprotein components including carbohydrates to form plaques.² Cell surface carbohydrates such as heparan sulfate (HS) can play important roles in $A\beta$ biology.^{3,4} HS is a class of highly sulfated glycans, consisting of disaccharide repeating units of glucosamine- α -1,4-iduronic acid/glucuronic acid.⁵ The *N*, 3-*O*, and 6-*O* of the glucosamine, and the 2-*O* position of the uronic acid can be sulfated, rendering HS a class of biomacromolecules with the highest density of negative charges in nature. HS can be displayed on the surface of neuronal cells as part of the heparan sulfate proteoglycans (HSPGs), which are known to be expressed at significantly higher levels in transgenic animal models of AD as well as in post-mortem human brain tissues from AD patients.^{3,4,6} Through binding of $A\beta$, cell surface HS can lead to the accumulation and the deposition of cytotoxic $A\beta$ on neuronal cells. Furthermore, HSPGs can mediate $A\beta$ internalization into the neuronal cells exacerbating neurotoxicity.^{7,8}

HS and heparin (HS with higher levels of sulfations and iduronic acid) can have potential beneficial effects on AD patients.⁹ In clinical studies, administration of heparin like compounds has been shown to relieve behavioral symptoms in animal models of Alzheimer disease as well as in multi-infarct

dementia human patients, although these compounds cannot be used directly for AD treatment due to concerns of potential bleeding side effects associated with anticoagulant activities of native heparin.^{10–12} HS mimics or nanoparticles can help mitigate cytotoxic $A\beta$ species,^{13,14} providing exciting leads for AD detection and therapy.

With the appreciation of the roles of HS in $A\beta$ biology, it is important to gain a deeper understanding of how the structural parameters of HS affect $A\beta$ interactions. Utilizing oligosaccharides isolated through degradation of heparin polysaccharides, Hung and co-workers showed that the HS tetrasaccharides can bind with $A\beta$ with a dissociation constant of 78 μ M as determined by NMR.¹⁵ No significant differences between the K_d values ($K_d \sim 40 \mu$ M) from hexamer to octadecamer were observed. In addition, the Radford and Middleton groups probed the importance of HS sulfation patterns.¹⁶ Utilizing HS polysaccharides chemically treated to selectively remove sulfates, they reported that the *N*-sulfate or 6-*O*-sulfate of glucosamine, but not the 2-*O*-sulfate of iduronate, was required

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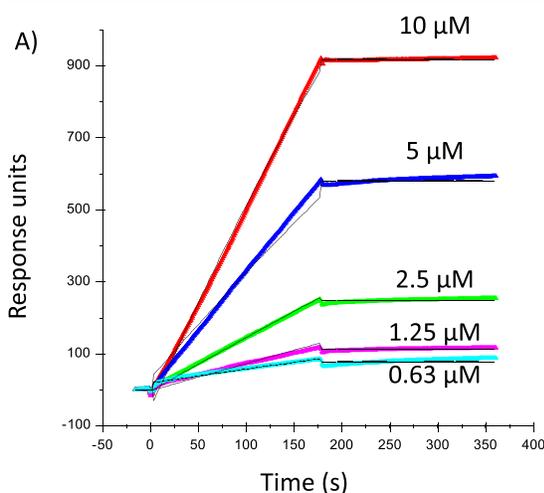
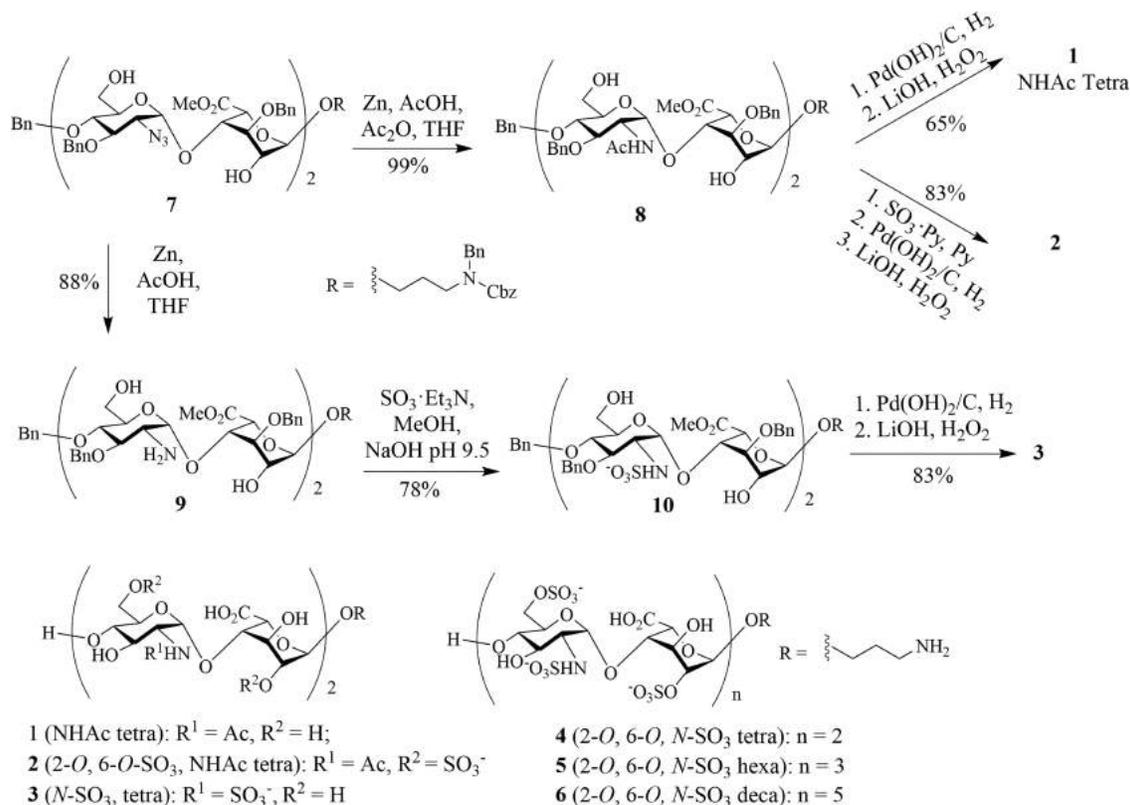
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Scheme 1. Structures of HS Oligosaccharides 1–6 and Synthesis of HS Tetrasaccharides 1–3



B)

Compound #	IC ₅₀	Binding affinity K _i
1	>200 μM	N.D.*
2	>200 μM	N.D.*
3	>200 μM	N.D.*
4	100 μM	600 nM
5	30 μM	178 nM
6	1.3 μM	7.7 nM

*N.D.: not detected.

Figure 1. (A) SPR sensorgrams of Aβ₄₀ binding with a heparin chip. Solutions of Aβ (0.63, 1.25, 2.5, 5, and 10 μM) were flowed over the heparin chip. On the basis of the responses, a K_d value of 15 nM for heparin-Aβ binding was calculated. (B) IC₅₀ values and inhibitory constants of various synthetic HS oligosaccharides binding with Aβ as determined by a solution/surface competition experiments against Aβ binding to heparin immobilized on an SPR sensor. IC₅₀ was calculated based on the protein binding signals from the sensorgrams with the addition of HS oligosaccharides in different concentrations. Solution based affinities (K_i) were calculated from IC₅₀ measured from SPR competition experiments using the equation: $K_i = \text{IC}_{50} / (1 + [C]/K_D)$; [C] = protein concentration used in the competition SPR; K_D is protein-heparin binding affinity.

for Aβ binding, indicating that selectivity in the interactions of HS with Aβ fibrils extends beyond general electrostatic complementarity. Other studies utilized fragments rather than full length Aβ peptides to probe HS/Aβ interactions.¹⁷

While the aforementioned studies have provided exciting insights into HS/Aβ interactions, a potential drawback in relying on HS isolated from nature is that chemical modification and degradation of heparin polysaccharides are

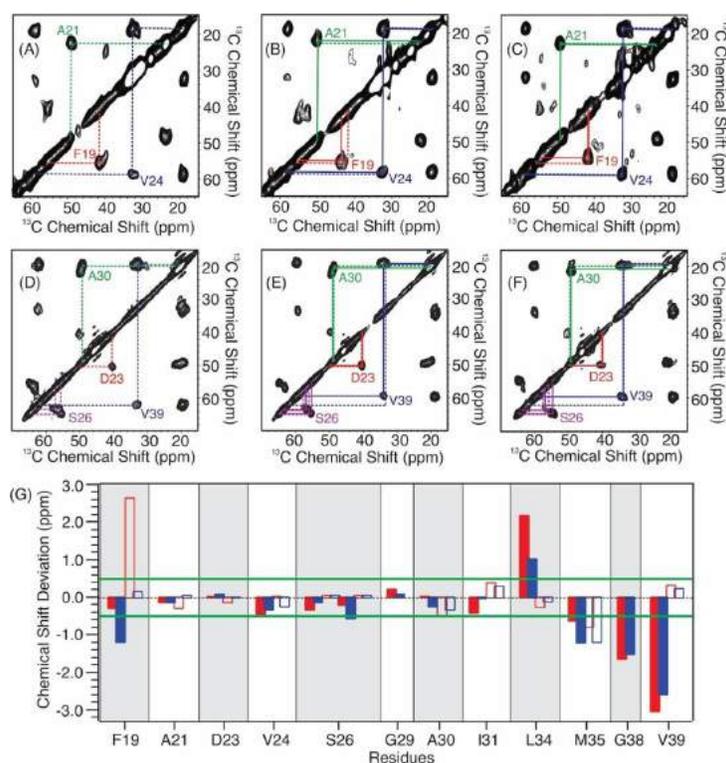


Figure 2. (A–F) Representative 2D ^{13}C – ^{13}C spin diffusion ssNMR spectra of (A, D) free $3\text{Q}\beta_{40}$ fibrils, (B, E) β fibrils with HS hexamer 5, and (C, F) β fibrils with HS decamer 6. The β sequences contain isotope-labeling at (A–C) F19, A21, V24, and G29 and (D–F) D23, S26, A30, and V39. The dashed lines in panels A and D highlight the intraresidue cross peaks in the free fibrils. The solid lines in panels B, C, E, and F show the intrasidue cross peaks in the HS-bound fibrils to emphasize the deviations in chemical shifts from the free fibrils (dashed lines in the same panel). (G) Plots of the residue-specific ^{13}C chemical shift deviations (i.e., $\Delta\delta = \delta_{\text{heparin-bound fibrils}} - \delta_{\text{free fibrils}}$) in the presence of HS hexasaccharide 5 (solid red columns, α ; open red columns, β) and decasaccharide 6 (solid blue columns, α ; open blue columns, β). The green lines highlight the 0.5 ppm threshold for significant chemical shift deviations based on the ^{13}C line widths in ssNMR spectra.

not site specific. In addition, with the inherent structure heterogeneity of the polysaccharide,¹⁸ the compounds subject to β binding were presumably still a mixture. Thus, we become interested in probing structural requirements for β binding using well-defined synthetic HS oligosaccharides, which include HS tetrasaccharides 1–4 with varying sulfation patterns, HS hexasaccharide 5,¹⁹ and decasaccharide 6.²⁰

The synthesis of HS tetrasaccharides 1–3 started from tetrasaccharide 7.²⁰ The 2-azido groups of tetrasaccharide 7 were reduced by zinc and acetic acid in the presence of acetic anhydride to provide 8 with two *N*-acetamides in 99% yield (Scheme 1), which was hydrogenated followed by saponification, generating tetrasaccharide 1 in 65% yield for the two steps. Alternatively, the free hydroxyl groups of 8 were sulfated with SO_3 ·pyridine in pyridine at 55 °C followed by global deprotection leading to tetrasaccharide 2 (83% overall yield for 3 steps). In order to prepare the *N*-sulfated tetrasaccharide 3, 7 was reduced with zinc and acetic acid without the addition of acetic anhydride in 88% yield. Sulfation of the two free amines in the resulting tetrasaccharide 9 was performed by first dissolving it in methanol with aqueous NaOH solution adjusting the pH to 10, which was followed by the addition of excess SO_3 · Et_3N complex to yield *N*-sulfated tetrasaccharide 10. Global deprotection of 10 produced the tetrasaccharide 3. Compounds 4–6 were prepared as previously described.^{19,20}

Surface plasmon resonance (SPR) assays were set up to measure HS- β binding to decipher structural impacts of HS on β interactions using a solution affinity assay.²¹ Biotinylated heparin polysaccharide was immobilized onto a streptavidin sensor chip, which was incubated with solutions of β with varying concentrations. From the resulting sensorgrams (Figure 1A), a K_d value of 15 nM for heparin- β binding was calculated. Solution/surface competition experiments were performed by incubating increasing concentrations of HS oligosaccharides with β before adding the mixture to the heparin sensor chip to measure the affinity of the synthetic HS oligosaccharides. The binding of the oligosaccharide with β would competitively inhibit the interaction of β with the heparin chip, thus reducing the responses observed on the SPR sensor. From these experiments, the IC_{50} values of the oligosaccharides were obtained (Figure 1B). K_d values were calculated from the SPR association and dissociation curves using Biacore BIAevaluation software. In a comparison of the tetrasaccharides 1–4, only tetrasaccharide 4²⁰ bearing three sulfates per disaccharide exhibited significant binding. As 4 has the highest density of negative charges, it suggests the importance of electrostatic interactions for HS tetrasaccharide- β binding. Increasing the length of the oligosaccharides from tetra- (4) to hexa- (5) and deca- (6) saccharide led to significant increases in affinity with a K_i value of 7.7 nM determined for decasaccharide 6. It suggests that with the same

number of negative charges per disaccharide, longer HS oligosaccharides have stronger binding with $A\beta$. This is in contrast to the trend observed using oligosaccharides obtained from the degradation of a naturally existing polysaccharide, where there were no significant changes in K_d values from hexamer to octadecamer.¹⁵ The difference may be due to the fact that naturally derived compounds are a mixture of glycans with various sulfation patterns, masking the effects caused by the changes in glycan length.

To obtain more structural insights into HS- $A\beta$ binding, we utilized the solid-state nuclear magnetic resonance (ssNMR) spectroscopy to investigate the residue-specific ^{13}C chemical shift perturbations of $A\beta$ fibrils upon interacting with HS hexasaccharide **5** and decasaccharide **6** respectively. Three isotopically labeled $A\beta$ sequences were synthesized with labeling sites (F19, A21, D23, V24, S26, G29, A30, I31, L34, M35, G38, and V39) that covered the typical fibrillar core segments of $A\beta_{40}$ fibrils.^{22–25} All fibril samples were prepared using the quiescent incubation protocols (i.e., resulting fibrils with a 3-fold symmetric quaternary structure, or 3Q fibrils).²⁶

The representative two-dimensional (2D) ^{13}C - ^{13}C spin diffusion ssNMR spectra (Figure 2A–F) highlighted the intramolecular cross-peaks of selective residues. Single sets of cross peaks were observed for most of the labeled sites, indicating well-defined local conformations in 3Q $A\beta$ fibrils with and without HS oligosaccharides. A few residues, such as S26, possessed local structural heterogeneity with multiple sets of cross peaks. Upon incubation with HS oligosaccharides, ^{13}C chemical shift perturbations were observed at specific residues (Table S1) with the hexasaccharide **5** and decasaccharide **6** giving comparable patterns of chemical shift changes suggesting similar binding modes for these two oligosaccharides (Figure 2G). Overall, the perturbations were most significant for residues F19, L34, M35, G38, and V39 and modest for S26, A30, and I31, while little changes were observed for A21, D23, V24, and G29.

The current ssNMR study provides new insights into the site-specific interactions between HS and $A\beta$ fibrils. Middleton and co-workers reported the site-specific interactions between an HS octasaccharide and the 3Q $A\beta$ fibrils by ssNMR, where large ^{13}C chemical shift perturbations were also observed for residues S26, A30, I31, and L34 with minimal perturbations for A21 and D23.¹⁶ On the other hand, the significant ^{13}C chemical shift perturbations at L34, M35, G38, and V39 observed in our study suggest an additional fibrillar segment that may bind to the HS oligomers. In the molecular structure of 3Q $A\beta$ fibrils, these residues are in close proximity with the segment A30–I32 of a neighboring $A\beta$ molecule,²⁶ where the latter residues showed significant chemical shift perturbations in the previous report.¹⁶ This cluster of residues located in the pore region of 3Q $A\beta$ fibrils is accessible to water molecules, where water can bind to this pore region and the polar interfaces consisting of terminal and interstrand loop residues possess similar spectroscopic and dynamic properties.²⁷ Thus, it is reasonable that the water-soluble HS oligosaccharides may enter and bind to the fibrillar pore. For residue S26, while multiple intraresidue cross peaks were observed in free 3Q $A\beta$ fibrils (Figure 2D), these cross peaks became more defined (e.g., two sets of peaks, Figure 2E) upon binding to the hexamer **5**, and one set of cross peaks became predominant in the presence of HS decamer **6** (Figure 2F). The local conformation of S26 was less ordered compared to the β -sheet segments (e.g., L17–A21 and A30–V36) in the unbound 3Q

$A\beta$ fibrils.²⁶ It is possible that the binding between HS oligomers and the fibrils rigidifies the complex structure, thus reducing the number of local conformers at the S26 site with the tighter binding decamer **6** leading to one major conformation.

$A\beta$ is known to be toxic to neuronal cells,²⁸ which can be a contributing factor to $A\beta$ pathology *in vivo*. As the synthetic HS oligosaccharides can bind with $A\beta$, their impacts on $A\beta$ toxicities were tested with the SH-SY5Y neuroblastoma cell line, a common model for neuronal screening.²⁹ SH-SY5Y cells were incubated with $A\beta$ in the presence of various HS oligosaccharides, and the viabilities of the cells were determined. As shown in Figure 3, HS oligosaccharides

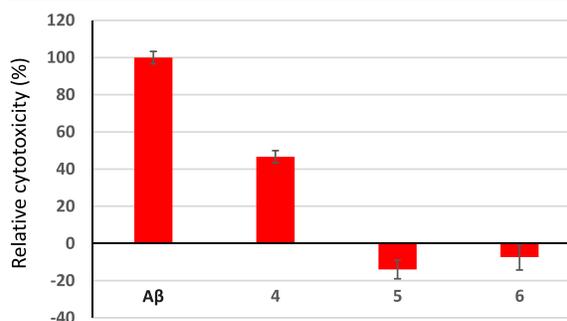


Figure 3. HS oligosaccharides can reduce the cytotoxicity of $A\beta$ to SH-SY5Y cells. SH-SY5Y cells (2×10^4) were incubated with or without $A\beta$ ($30 \mu\text{M}$) for 48 h. The numbers of viable cells were determined via the MTS cell viability assay. The change of the cell number due to $A\beta$ was determined. Cells were then incubated with $A\beta$ ($30 \mu\text{M}$) in the presence of HS **4**, **5**, and **6** ($60 \mu\text{M}$), respectively. The numbers of viable cells were determined by the MTS cell viability assay after 48 h, respectively. The changes in cell numbers were calculated compared to cells cultured in media only. Relative cytotoxicity values were calculated based on the following formula: (changes of the number of cells with HS/ $A\beta$)/(changes of the number of cells with $A\beta$ only) $\times 100\%$.

reduced $A\beta$ toxicities with the hexasaccharide **5** and decasaccharide **6** providing complete protection under the experimental conditions. The abilities of the HS oligosaccharides to mitigate $A\beta$ toxicity could be because HS binding shielded the toxic $A\beta$ from interacting with HS on the cell surface.

In conclusion, HS tetrasaccharides with varying sulfation patterns have been synthesized. The binding of these tetrasaccharides as well as synthetic HS hexa- and decasaccharides with $A\beta$ have been measured by SPR. The highly sulfated tetrasaccharide **4** exhibited the strongest binding with $A\beta$ among the tetrasaccharides tested. Increasing the length of the glycan from tetrasaccharide to decasaccharide significantly enhanced the affinity with $A\beta$. ssNMR studies demonstrated that HS binding could induce significant chemical shift perturbations in $A\beta$ fibrils, with major changes observed within the C-terminus residues. The HS oligosaccharides could reduce the cellular toxicities induced by $A\beta$. As these synthetic HS oligosaccharides lack the 3-O sulfation, they should not have any anticoagulation activities. Thus, HS oligosaccharides can be leads for the further development of HS glycan-based mimetics to treat Alzheimer disease.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acscchembio.0c00904>.

Detailed experimental procedures, preparation, and characterization of the products (PDF)

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Author Contributions

[#]Equal contribution to this work.

Notes

The authors declare no competing financial interest.

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