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Aβ(1-42) Assembly in the Presence of *scyllo*-Inositol Derivatives: Identification of an Oxime Linkage as Important for the Development of Assembly Inhibitors

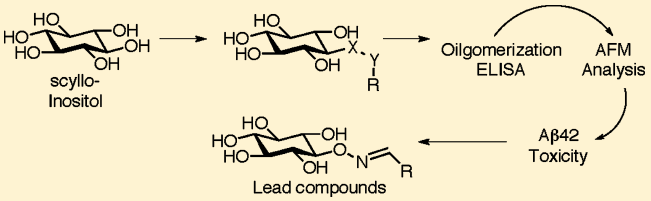
J. E. Shaw,[†,⊥] J. Chio,[‡,⊥] S. Dasgupta,[‡,⊥] A. Y. Lai,[†] G. C. H. Mo,[§] F. Pang,[†] L. A. M. Thomason,[†] A. J. Yang,[∥] C. M. Yip,[§] M. Nitz,*[,‡] and J. McLaurin*[,†]

[†]Department of Laboratory Medicine and Pathobiology, [‡]Department of Chemistry, and [§]Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada

[∥]Department of Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, Maryland, United States

Supporting Information

ABSTRACT: To identify a lead skeleton structure for optimization of *scyllo*-inositol-based inhibitors of amyloid-beta peptide (Aβ) aggregation, we have synthesized aldoxime, hydroxamate, carbamate, and amide linked *scyllo*-inositol derivatives. These structures represent backbones that can be readily expanded into a wide array of derivatives. They also provide conservative modifications of the *scyllo*-inositol backbone, as they maintain the display of the equatorial polar atoms, preserving the stereochemical requirement necessary for maximum inhibition of Aβ(1-42) fiber formation. In addition, a reliable work plan for screening derivatives was developed in order to preferentially identify a backbone(s) structure that prevents fibrillogenesis and stabilizes nontoxic small molecular weight oligomers, as we have previously reported for *scyllo*-inositol. In the present studies, we have adapted a high throughput ELISA-based oligomerization assay followed by atomic force microscopy to validate the results screen compounds. The lead compounds were then tested for toxicity and ability to rescue Aβ(1-42) induced toxicity in vitro and the affinity of the compounds for Aβ(1-42) compared by mass spectrometry. The data to suggest that compounds must maintain a planar conformation to exhibit activity similar to *scyllo*-inositol and that the oxime derivative represents the lead backbone for future development.



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scyllo-Inositol, a potential therapeutic compound for Alzheimer's disease, has been shown to inhibit Aβ(1-42) fibrillogenesis in vitro,[1] stabilize cell-derived small molecular weight oligomers,[2] reduce amyloid plaque load and neuroinflammation, and ameliorate cognitive deficits in vivo.[2,3] These combined studies demonstrated that *scyllo*-inositol stabilizes low molecular weight oligomers of Aβ that are nontoxic and readily removed from and/or degraded within the central nervous system. *scyllo*-Inositol is the most potent inositol, with a single epimerization to form *myo*-inositol yielding a less active compound in vivo and in vitro, suggesting that efficacy is highly dependent on inhibitor stereochemistry.[3] *scyllo*-Inositol-induced changes to the peptide assembly of Aβ(1-42) are very sensitive to structural perturbations of *scyllo*-inositol such as the number and orientation of the hydroxyl groups.[2] These structure–function studies revealed that even the most conservative single hydroxyl substitution led to a decrease in compound potency with only 1-deoxy-1-fluoro-*scyllo*-inositol retaining properties similar to the parent *scyllo*-inositol.[4] However, the introduction of two hydrophobic substituents as in 1,4-dimethyl-*scyllo*-inositol also provided a potent compound which alters the Aβ(1-42) aggregation pathway.[4] When administrated orally, 1,4-dimethyl-*scyllo*-inositol inhibited Aβ(1-42) assembly, decreased central nervous system Aβ load, and rescued mice from Aβ-induced memory impairment.[5] Here we choose to synthesize a series of compounds that maintain the polar periphery of the inositol ring while allowing the exploration of potential flanking binding sites. Four linkage chemistries to the *scyllo*-inositol-Aβ structure were explored which have led to new compounds which reduce the toxicity of Aβ(1-42). *scyllo*-Inositol derivatives were synthesized that contain an amide, oxime, carbamate, or hydroxamate linkages to alkyl groups and aryl rings. These structures give insight into which linkage chemistry is best suited for further development.

The immediate challenge was to develop a reliable, sensitive, and timely workflow for identifying promising *scyllo*-inositol derivatives. Our criteria for successful leads are the preferential stabilization of low molecular weight Aβ oligomers, inhibition of fibril formation, and rescue of Aβ(1-42) toxicity in cell culture. To date, screening protocols have targeted inhibition of fibrillogenesis and/or oligomerization more generally,[2,6−11] with the majority of high-throughput assays measuring amyloid protein aggregation by thioflavin T (ThT) fluorescence[6,7,9] or enzyme-linked immunosorbent assays (ELISA).[8,10,11] The potential caveats of using ThT fluorescence to measure compound-induced Aβ(1-42) assembly are that Aβ oligomers can bind ThT, ThT may

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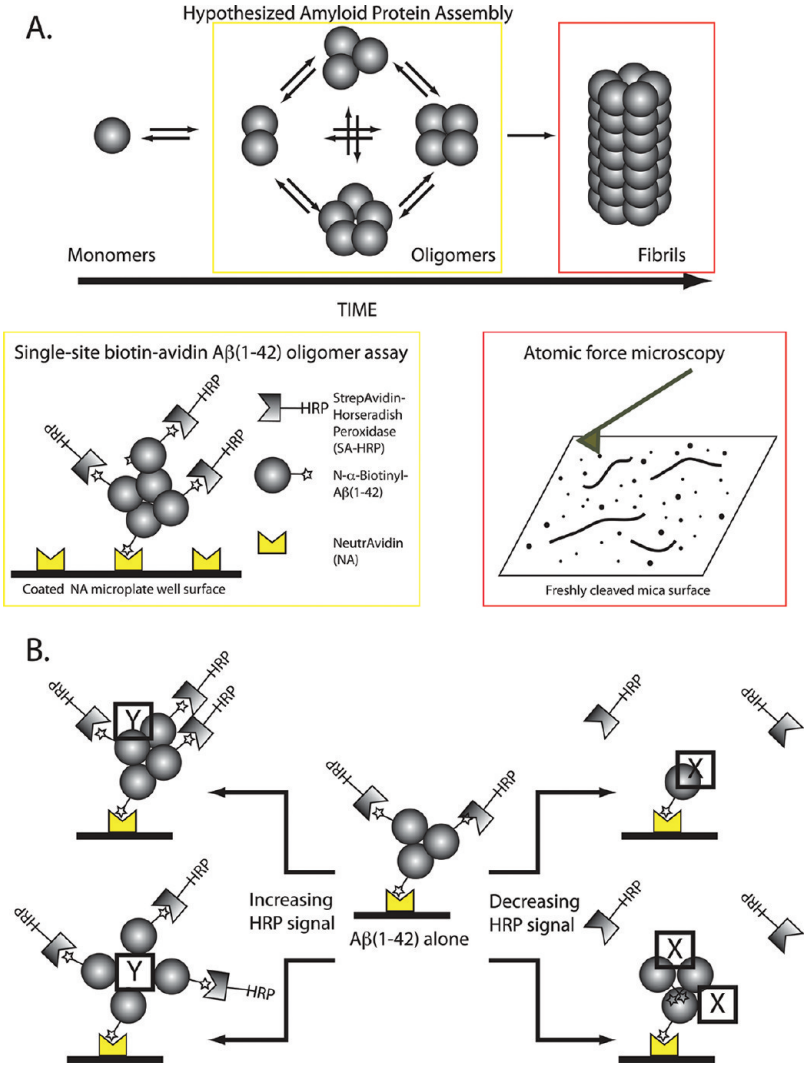

Figure 1. (A) Two-step workflow combined with biotin–avidin oligomer assay screening with atomic force microscopy. (B) Schematic for the oligomer assay.

compete for potential inhibitor binding sites, and inner filter effects from strongly absorbing compounds.[12] To address these concerns, we have developed a two-step workflow for screening compounds by combining a biotin–avidin Aβ(1-42)-based oligomer assay[10,13] with a morphological analysis for fiber formation by atomic force microscopy (AFM) (Figure 1). The inclusion of a structural analysis is critical, as small molecules have been shown to fall into three classes, those that inhibit oligomerization but not fibrillization, those that inhibit fibrillization but not oligomerization, or those that inhibit both.[11] AFM is the preferred method over electron microscopy for visualizing Aβ oligomers, as it can resolve sample heterogeneity of the peptide assembly with higher resolution without potential artifacts due to sample preparation.[14] The lead compounds identified were then assayed for reducing Aβ(1-42) toxicity in vitro, to confirm the compounds and oligomers formed in the presence of the compounds were not toxic. It is well documented that Aβ oligomers are toxic to neurons and therefore compounds that favor the stabilization of Aβ oligomers may enhance toxicity as was reported for naphthalene sulfonates and some N-methylated peptides.[15,16]

In the present studies, we validated the biotin–avidin oligomerization assay using inositol stereoisomers that we have previously employed in Aβ(1-42) aggregation assays as well as a structurally unrelated control, epigallocatechin gallate (EGCG).[1,17–19] Significant compound-induced oligomerization enhancement or inhibition from the assay (relative to Aβ(1-42) alone) can be correlated with morphological changes in Aβ(1-42) structures, as imaged by AFM. In addition, the oligomerization activities of the compounds were found to be nonlinear with respect to compound concentration and the Aβ(1-42)-to-compound ratio. By incorporating a range of compound concentrations in the ELISA, we have identified oligomerization profiles that predict stabilization of low or high molecular weight oligomers versus fibres of Aβ(1-42).

Among the compounds tested in this study, we found that *scyllo*-inositol, and the oxime functionalized *scyllo*-inositol, significantly enhanced overall oligomerization of Aβ(1-42) relative to the control and prevented Aβ(1-42) fiber formation. These results suggest that further development of oxime derivatives may lead to more potent stabilizers of low molecular weight Aβ(1-42) oligomers.

RESULTS AND DISCUSSION

Validation of Single-Site Biotin–Avidin Aβ(1-42) Oligomer Assay. We have modified a previously published Aβ oligomer assay.[10,13] The assay uses capture and detection reagents that bind biotin producing an increased signal when oligomeric

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Aβ(1-42) complexes are formed in the assay (Figure 1A). It has been shown that N-terminal biotinylation of Aβ(1-42) does not significantly alter the assembly process.[20,21] Fibrillization is less likely to occur than oligomerization in the assay because of the low final biotin-Aβ(1-42) concentration of 14 nM.[10] We assayed previously tested inositol stereoisomers and carbohydrates over a range of concentrations to validate this assay at defined compound: Aβ(1-42) ratios.[1,22]

Both *scyllo*- and *myo*-inositol gave statistically significant increases Aβ(1-42) oligomerization over the concentration range as shown by the ELISA and inhibited Aβ(1-42) fibrillization as shown by AFM (Figure 2A and B) while (L) *chiro*-inositol showed statistically significant decreases in oligomerization and increased fibril growth (Figure 2C). This is consistent with previous reports that *scyllo*- and *myo*-inositol induced a β-structural transition measured by circular dichroism,[1] enhanced ThT fluorescence relative to Aβ(1-42) alone,[23] and inhibited fibril growth as seen using negative-stain electron microscopy while L-chiro-inositol had no effect.[1] These results suggested that a significant increase in compound-induced oligomerization indicated by the ELISA is a good predictor of efficacy in modulating Aβ(1-42) fibrillogenesis. To validate this hypothesis, we screened additional compounds that we have previously reported in the literature to modulate the Aβ(1-42) aggregation cascade. *epi*-Inositol, a known fibril inhibitor,[1] exhibits an increasing concentration dependent oligomerization profile while D-*chiro*-inositol, galactose, mannose, and sucrose, which do not inhibit fibril formation,[1,22] exhibit either decreasing oligomerization or no change in the oligomerization profiles (Figure S1). Two polyphenols, EGCG and a related analogue, taxifolin, were also assayed. EGCG has been shown to inhibit Aβ(1-42) fibrillization by accelerating oligomer aggregation.[19] The oligomerization profile of EGCG showed a sharp increase in Aβ(1-42) oligomerization at 1:5 peptide-to-compound molar ratio (Figure S2) while taxifolin showed no change in Aβ(1-42) oligomerization. These results agree with the AFM images of Aβ aggregation, which showed that EGCG significantly alters the fibrillization process while taxifolin does not (Figure S2).

Evaluation of Aβ(1-42) Assembly in the Presence of *scyllo*-Inositol Derivatives. In our previous studies, we showed that *scyllo*-inositol modulates Aβ(1-42) fibril assembly.[1,4] In the present study, we investigated 17 novel *scyllo*-inositol derivatives with varying linkages between *scyllo*-inositol and various side chains in order to identify a structural backbone for future compound development (Figure 3). The rationale for synthesis of these compounds originated from the observation that only minimal perturbations around the hydrophilic *scyllo*-inositol periphery resulted in compounds active in the Aβ(1-42) aggregation cascade. Simple deoxygenation or a single methylation greatly reduced the potency of the compounds in preventing Aβ(1-42) assembly. Interestingly, introduction of two methyl groups at the 1 and 4 positions yielded a derivative that increased Aβ(1-42) oligomerization and had efficacy in in vivo mouse models.[5] Thus, more hydrophobic *scyllo*-inositol derivatives may represent potential inhibitors of Aβ(1-42) assembly. The new *scyllo*-inositol derivatives are considerably more hydrophobic but vary with respect to their covalent linkage between *scyllo*-inositol and the side chains. The different linkages allow determination of the best combination of polar atoms at the *scyllo*-inositol periphery and the optimal orientation of the side chains to maximize the influence of the compound on Aβ(1-42) assembly.

Chemical Synthesis. The compounds were synthesized as outlined in Figure 3. Briefly, compounds **1** and **2** can be produced in a three-step procedure from *myo*-inositol.[24] The triflate is readily displaced with azide to give compound **14**. Removal of the protecting groups from compound **14** with 95% TFA gave the azide derivative **16**. Hydrogenation of the azide in compound **14** followed by deprotection and acylation led to compounds **15**, **17**−**20**. Introduction of the hydroxylamine functionality to the inositol ring of **1** proved challenging with the best yields being obtained under concentrated reaction conditions in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) using *N*-hydroxyphthalimide as the nucleophile to give compound **3**. Global deprotection of **3** gave moderate yields of compound **4**, which reacts readily with aldehydes to give the oximes **5**−**11**, exclusively as the E isomers. Alternatively, the pthalimido group of **3** was selectively removed and upon treatment with benzoyl chloride the dibenzamido compound **12** was produced. Sequential methanolysis followed by acid hydrolysis gave the benzamido-*scyllo*-inositol **13**.

Antiaggregation Properties of Novel Compounds. We initiated our analyses by building scaffold molecules based on azide, amide, and carbamate *scyllo*-inositol derivatives (**15**−**20**) (Figure 4). The azide appended *scyllo*-inositol derivative, **16**, demonstrated an aggregation profile similar to that observed with the parent *scyllo*-inositol (Figure 2A), showing a concentration dependent increase in oligomerization (Figure 4A). The corresponding AFM images show both protofibrillar and oligomeric structures, suggesting that compound **16** more weakly stabilizes Aβ(1-42) oligomers when compared to the parent *scyllo*-inositol. The 1-deoxy-1-amino-*scyllo*-inositol (compound **15**) showed minimal differences from the control in the oligomerization assay and AFM investigation confirmed no differences between Aβ(1-42) structure in the presence or absence of this compound (Figure 4B). However, both the acetyl (**17**) and the pivaloyl (**18**) *scyllo*-inositol derivatives gave aggregation profiles with reduced aggregation at low concentrations, and subsequently increasing oligomerization at higher concentrations (Figure 4C,D). The AFM images in the presence of compounds **17** and **18** showed a mixture of oligomeric, short fibres and a few protofibrils. The phenyl amide linked *scyllo*-inositol derivative (**19**) did not show any modulating effects with oligomerization or fibrillation (data not shown). These results suggest that introducing groups via an amide linkage to *scyllo*-inositol is moderately effective for alkyl substituents but is not optimal for larger more hydrophobic aromatic rings.

Given the low activity of the alkyl amide derivatives (**17**, **18**) and the lack of activity with the phenyl derivative (**19**), we hypothesized that the amide did not present the new hydrophobic side chains in an optimal orientation. We investigated the introduction of phenyl substituents linked via carbamate, hydroxamate, and oxime linkages, which will provide different orientations of the phenyl substituent relative to the inositol ring. While the oligomerization profile of the carbamate derivative (**20**) reached a maximum at 1:5 molar ratio (up to 27%) and subsequently decreased at higher molar ratios, it did not inhibit fibrillization (data not shown). The hydroxamate derivative (**13**) gave reduced oligomerization profile in the ELISA, and AFM imaging showed that this derivative did not inhibit fibril growth (data not shown). In the Aβ(1-42) ELISA, we found that the phenyl-oxime derivative (**5**) was able to increase oligomerization approximately up to

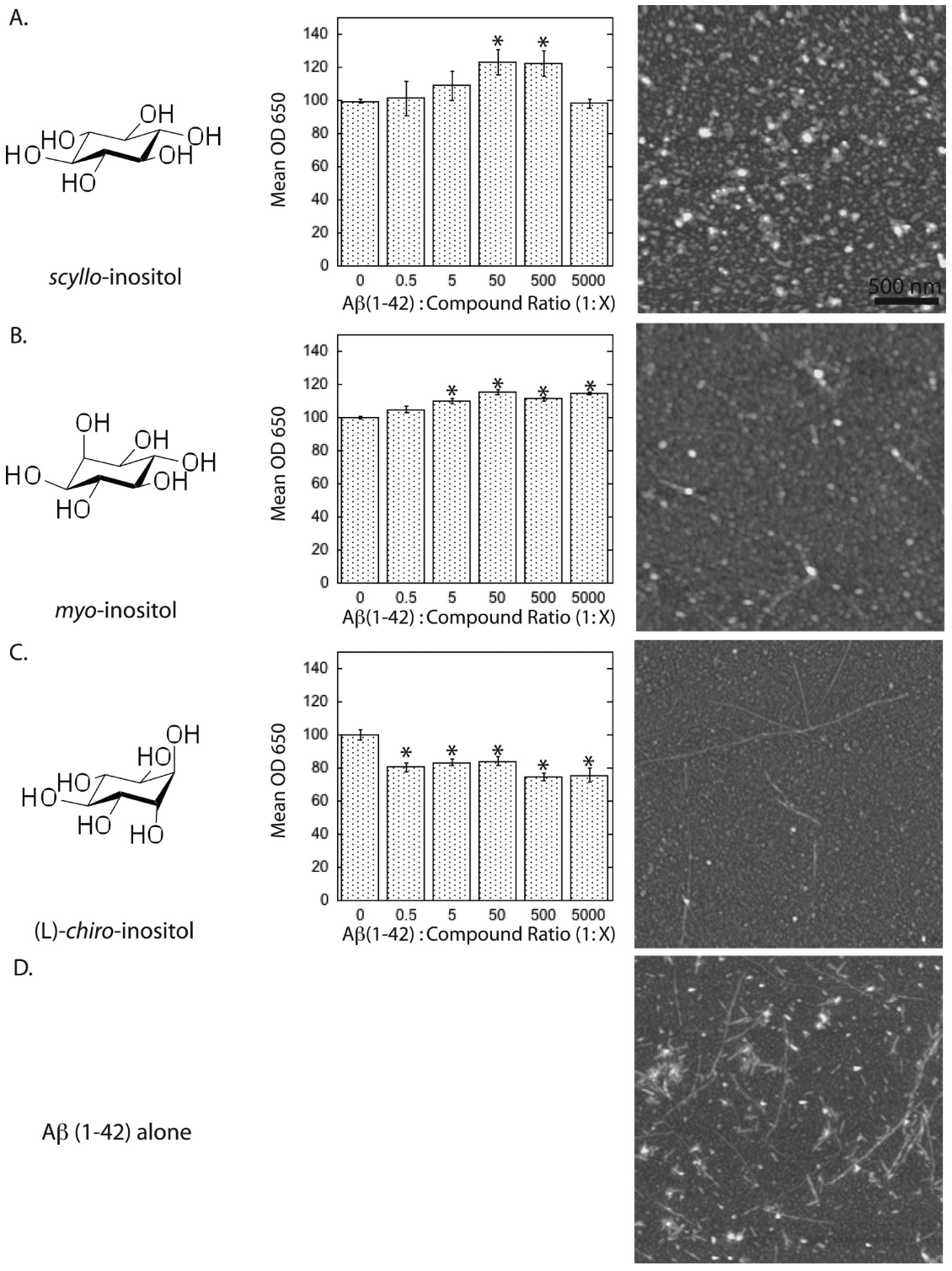

Figure 2. Chemical structures (left column) of *scyllo*-inositol, *myo*-inositol, and (L) *chiro*-inositol assayed by A*β*(1-42) oligomer assay (middle column) and atomic force microscopy (right column). For the oligomer assay, 15 nM of biotinylated A*β*(1-42) was incubated with or without the compounds (7.5 nM to 75 μM) for 3 days at 37 °C. The degree of accumulated aggregation was determined by reading optical density and measurements are normalized to the average intensity of A*β*(1-42). Asterisk (*) represents $p < 0.0033$. For studying fiber assembly, 45 μM of A*β*-(1-42) was incubated with or without the compounds (22.5 mM) at 37 °C before performing AFM imaging. The data are representative tapping mode AFM images acquired in air. Image size: 2 μm × 2 μm with a 20 nM height scale. The bar is 500 nm.

54% at 1:5000 molar ratio and significantly increased fibril assembly of A*β*(1-42) as determined by AFM (Figure 5A). These results suggested that the linkage between *scyllo*-inositol and the side chain dramatically influences the interaction with A*β*(1-42).

Energy-minimized conformations of the phenyl substituted *scyllo*-inositol derivatives generated by molecular modeling **19**, **20**, **13**, and **5** revealed that the inositol ring and the phenyl ring are coplanar for the oxime derivative (**5**) (Figure 6). In the amide (**19**) and carbamate (**20**), the phenyl ring is twisted with

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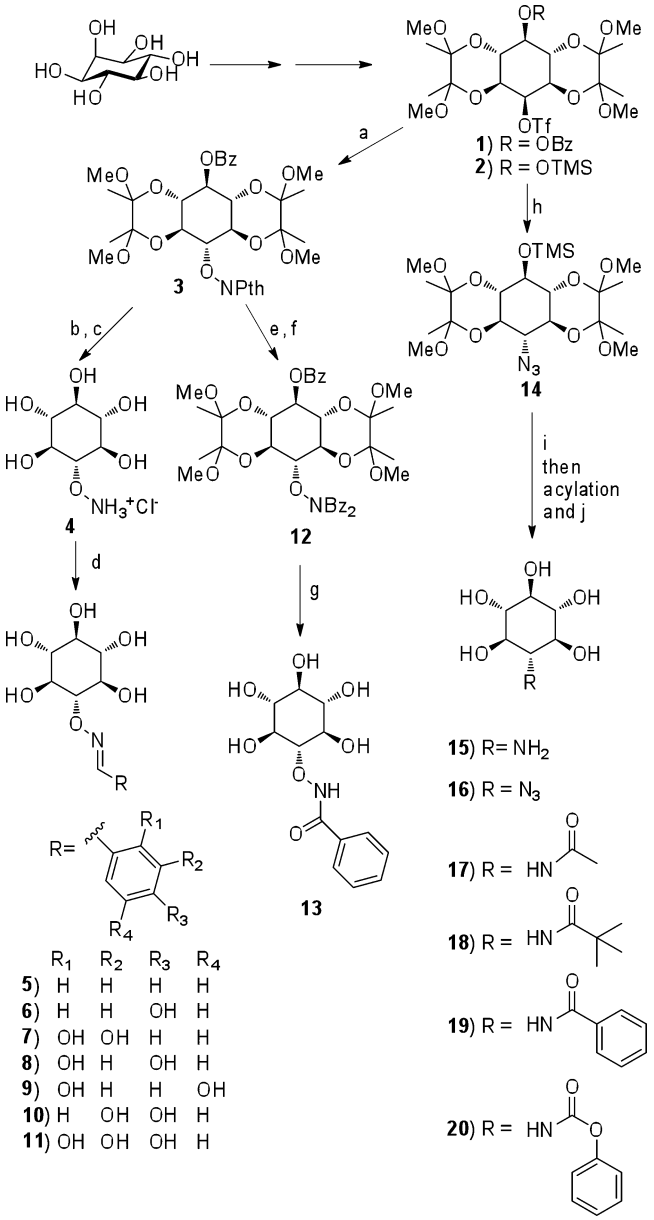

Figure 3. Preparation of *scyllo*-inositol derivatives. Syntheses of compounds **1** and **2** can be found in ref 22 and the Supporting Information. (a) PthN−OH, Et_3N, DMPU, **1**, 76%; (b) 95% TFA, 98%; (c) (i) $MeNH_2$/MeOH, (ii) conc. HCl, 59%; (d) aldehyde, THF/H_2O 1:1, 65−90%; (e) 2 M $MeNH_2$ in MeOH, 86%; (f) BzCl, Et_3N, CH_2Cl_2, 74%; (g) (i) NaOMe/MeOH; (ii) 95% TFA, 52% over two steps; (h) NaN_3, DMF, **2**, 60 °C, (i) H_2, Pd/C, EtOH; (j) 95% TFA.

respect to the inositol ring. The benzamido derivative (**13**) adopts a puckered structure enforced by a strong hydrogen bond between the *scyllo*-inositol hydroxyl group and the carbonyl. Given the differences observed in the activity of the compounds toward Aβ(1-42) oligomerization and fibrillization, the optimal functional group for substitution of the *scyllo*-inositol skeleton is an oxime. This linkage chemistry introduces the minimal amount of steric bulk to the periphery of the inositol and also orients the oxime substituent in a coplanar geometry with the inositol ring. This conformation is similar to those of flat aromatics such as the polyphenols, which are known inhibitors of fiber formation.[25]

While *scyllo*-inositol and phenyl-oxime-*scyllo*-inositol derivative (**5**) increase Aβ(1-42) oligomerization as shown ELISA, the concentration dependence of oligomerization suggest these compounds may affect Aβ(1-42) at different points of the assembly pathways. *scyllo*-Inositol shows increases in Aβ(1-42) oligomerization profile at lower compound concentrations than compound **5** and completely inhibits fiber formation by inducing globular aggregates in uniform sizes that adsorb homogeneously onto the mica surface. Phenyl-oxime-*scyllo*-inositol (**5**) also produces an increase in oligomerization profile at high concentration, but AFM shows Aβ(1-42) fibers (Figure 5A). Given the promise of the oligomerization activity of compound **5**, we have targeted the oxime derivatives for further optimization.

The oxime derivatives are similar in geometric structure to polyphenols, we hypothesized that the general principles identified for structure−function activity of polyphenol−Aβ interactions may also apply to these *scyllo*-inositol derivatives.[26,27] Previous polyphenol studies have shown that a minimum of two aryl rings are required for Aβ(1-42) binding, with one ring being a phenolic group which contains at least one hydroxyl substitution and the length of the linker is critical for activity.[27] We therefore synthesized a series of compounds with increasing number and positioning of hydroxyl groups around the phenyl-*scyllo*-inositol backbone with an oxime linkage, compounds **6**−**11**.

A single substitution of a hydroxyl group at any position on the ring gave little change in the oligomerization assay, and no differences were detected by AFM for the *p*-hydroxy substituted oxime (**6**) (Figure 5B). In contrast, compound **8** with two hydroxyl groups on the phenyl ring at positions 1 and 3 exhibited a similar pattern to the parent phenyl-oxime derivative, with a decrease in fiber formation as resolved by AFM (Figure 5C). Altering the distribution of the two hydroxyl groups across the phenyl ring, compounds **7**, **9**, and **10**, decreased activity in both the oligomerization and AFM assays (data not shown). Surprisingly, the trihydroxyphenyl-oxime-*scyllo*-inositol, derivative **11**, showed a decrease in Aβ(1-42) oligomerization at the highest concentrations and multiple Aβ oligomeric species were observed by AFM (Figure 5D). These results suggest that mimicking the polyphenol structures does little to improve the potency of the *scyllo*-inositol derivatives.

We have identified a number of compounds that alter Aβ(1-42) aggregation from our screening protocol; however, it is unclear whether these oligomers represent toxic or nontoxic oligomers, as was previously shown for *scyllo*-inositol-Aβ(1-42) oligomers.[1,4,28] We identified compounds **5**, **6**, **8**, **11**, **16**, and **17** as potential compounds for further development; we therefore examined the compound and the compound Aβ(1-42)-induced toxicity of SH-SY5Y cells in culture (Figure 7). Of the amine-linked derivatives, only 1-deoxy-1-azide-*scyllo*-inositol (**16**) rescued Aβ(1-42)-induced toxicity (Figure 7A), whereas derivative **17** alone induced as much toxicity as Aβ alone (Figure 7B). Of the oxime-linked derivatives, only the phenyl-oxime (**5**) derivative rescued Aβ(1-42)-induced toxicity (Figure 7C) while the remaining compounds had no effect on toxicity. These results confirm that compound-stabilized oligomers may represent toxic intermediates and must therefore be prescreened in all drug development programs.

To investigate the relative affinity of compound-Aβ(1-42) binding interaction, we analyzed the binding of Aβ42 with the *scyllo*-inositol derivatives by a hybrid Orbitrap high-resolution mass spectrometry.[29] Electrospray ionization (ESI) has an advantage to study weak noncovalent ligand−protein interaction

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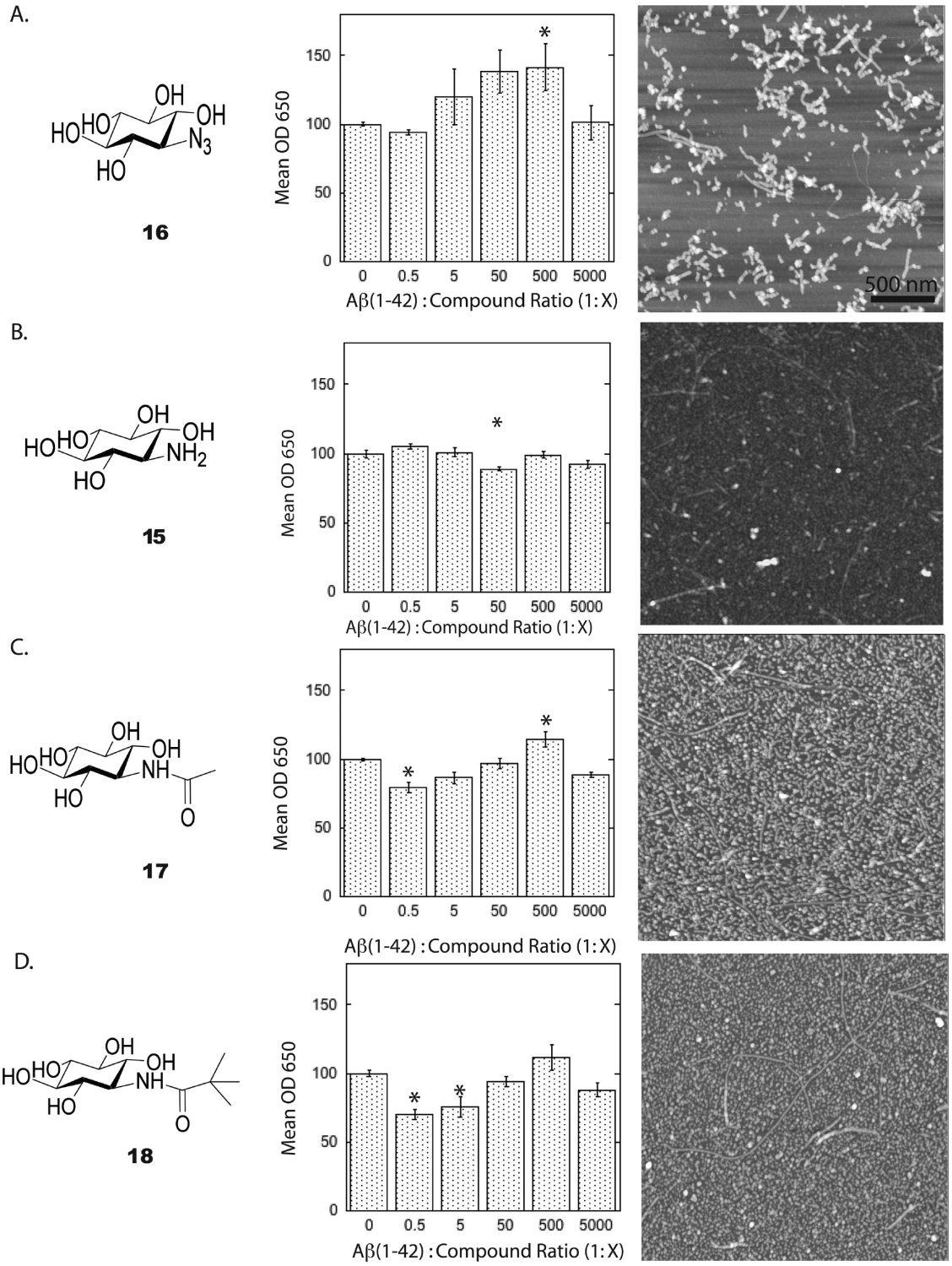

Figure 4. Chemical structures of azide and amide linked *scyllo*-inositol derivatives examined using the oligomer assay and atomic force microscopy. Compound **16** (A) exhibited an oligomeric profile similar to *scyllo*-inositol and stabilizes mostly oligomeric species, and compound **15** (B) showed no significant difference in oligomerization in comparison with Aβ(1-42) alone. While compound **17** (C) and **18** (D) derivatives demonstrate an attenuated profile that resulted in mixture of Aβ(1-42) species. Asterisk (*) represents $p < 0.0033$. The data are representative tapping mode AFM images acquired in air. Image size: 2 μm × 2 μm with a 20 nM height scale. The bar is 500 nm.

because the electrostatic component of interaction is enhanced in the gas phase, and this type of mass spectrometry instrumentation is complementary to other biophysical methods.[30] Since compounds **5** and **16** rescued Aβ(1-42)-induced toxicity, we focused our analyses on these compounds. Aβ(1-42) by high mass accuracy ESI linear LTQ Orbitrap mass spectrometer demonstrated a +5 charge "mass envelope" of unbound Aβ-(1-42) at m/z = 903.6 (Figure 8A). The charge state distribution of unbound Aβ(1-42) by ESI mass spectrometer is directly related to the pH and solvent content. Compounds **5** (m/z = 959.99; normalized ion intensity 5.92E3; Figure 8B) showed a strong binding to Aβ(1-42) with mass envelopes

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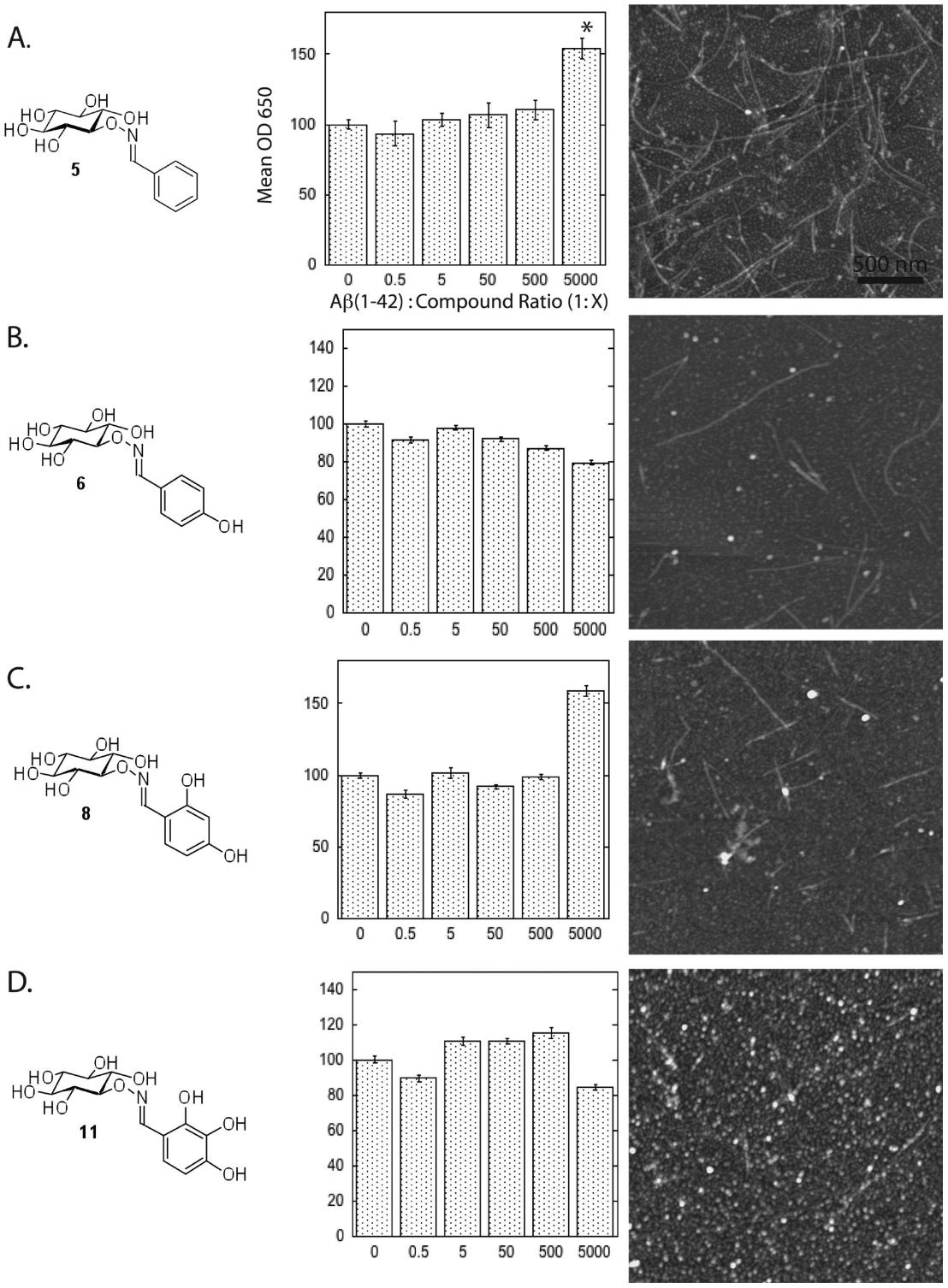

Figure 5. Chemical structures of the oxime linked phenyl-*scyllo*-inositol derivatives with increasing number of ring hydroxyl groups examined using the oligomer assay and atomic force microscopy. Phenyl-*scyllo*-inositol derivative demonstrated an increasing oligomeric profile and promotion of Aβ(1-42) fibers (A). However, addition of one (B) or two (C) hydroxyl groups on the ring decreased fiber formation. The 1,2,3-hydroxyl-*scyllo*-inositol derivative demonstrated a profile that was similar to *scyllo*-inositol by attenuated in fold effect, which stabilized oligomeric Aβ(1-42) species. Asterisk (*) represents $p <$ 0.0033. The data are representative tapping mode AFM images acquired in air. Image size: 2 μm × 2 μm with a 20 nM height scale. The bar is 500 nm.

detected at the predicted m/z ratio. In contrast, derivative **16** and Aβ(1-42) complex is less stable in the gas phase as demonstrated by the broad mass spectrum and low normalized ion intensity (3.11E3) (Figure 8C). These results demonstrate that changes in oligomerization detected by biophysical assays are directly correlated to direct binding of *scyllo*-inositol derivatives to Aβ(1-42).

In this study, we have validated the specificity and sensitivity of a single-site biotin−avidin Aβ(1-42) oligomer assay against *scyllo*-inositol related compounds reported in the literature.

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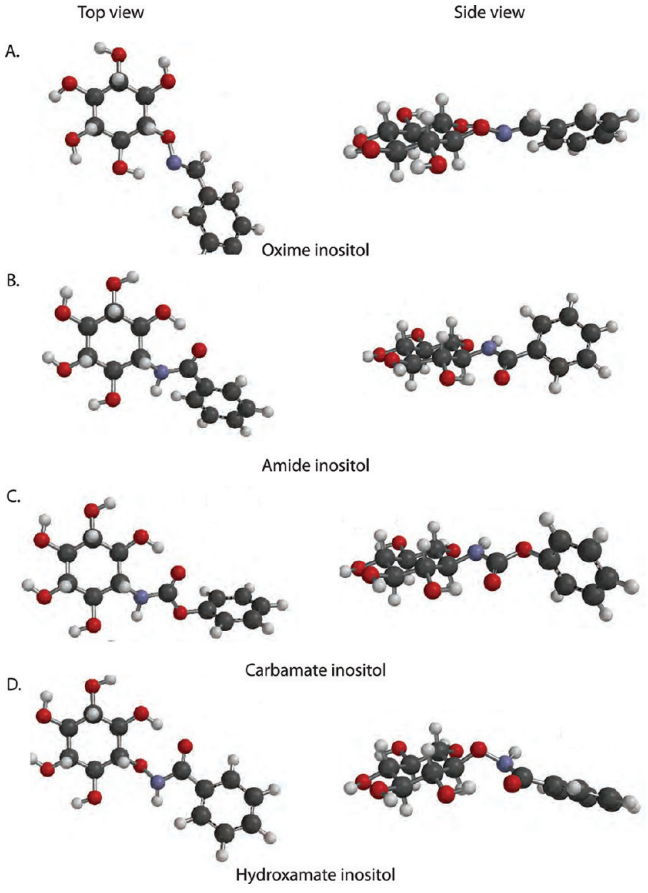

Figure 6. Molecular models of *scyllo*-inositol derivatives: (A) top view and (B) side view.

This assay will be used as a high-throughput screening tool for effective fibril modulators as our results demonstrate that compounds that significantly increase oligomerization are likely modulators of fibril formation. Since similar oligomerization profiles produce different structural aggregates, it is essential to use AFM to further validate the plate outcomes. Once fibril modulators have been identified, it is important to assay the influence of the modulating compound on Aβ(1-42) toxicity, as compounds were identified that modulated fibril formation but did not reduce Aβ(1-42) toxicity. Furthermore, we have shown that it is possible to obtain qualitative information on the relative affinities of the lead compounds for Aβ(1-42) using mass spectrometry. Lastly, in the present study, a new potential *scyllo*-inositol based fibril modulator has been identified. This compound contains the chemically versatile oxime linkage. Using the synthetic strategy outlined here, a large library of oximes can be rapidly generated by condensing the hydroxylamine substituted *scyllo*-inositol skeleton with commercially available aldehydes and ketones.

METHODS

Details of the chemical synthesis can be found in the Supporting Information.

Peptide Synthesis. Aβ(1-42) was synthesized by solid-phase 9H-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl (Fmoc) chemistry by the Hospital for Sick Children's Biotechnology Centre (Toronto, ON, Canada). The peptide was dissolved in DMSO as a stock solution at 10 mg/mL (or 2.2 mM) and stored at −20 °C until use. Biotin-Aβ(1-42) was commercially available from AnaSpec Inc. (Fremont, CA). It was dissolved in hexafluoroisopropanol (HFIP) as a stock solution of

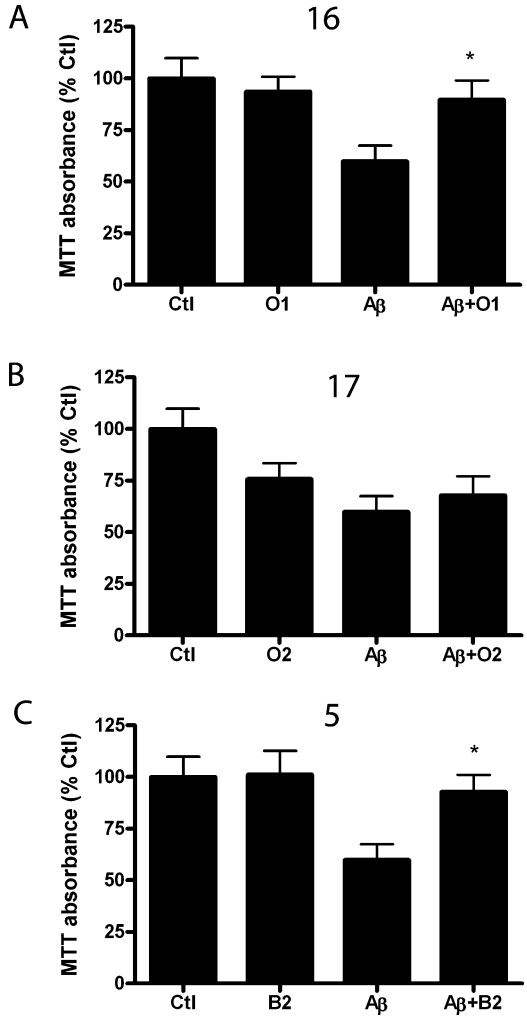

Figure 7. Compound-induced rescue of Aβ(1-42) induced-toxicity in vitro. Aβ(1-42) was incubated in the presence or absence of *scyllo*-inositol derivatives, followed by incubation with SH-SY5Y cells for 48 h. Compound **16** (A) rescued toxicity, while compound **17** did not (B). The phenyl-oxime-**5** (C) derivative rescued Aβ(1-42)-induced toxicity. Toxicity was measured using MTT assay, and bars represent the standard error of the mean of $n = 3$ separate experiments. *$p < 0.05$.

1 mg/mL (0.22 mM) and stored at −20 °C until use. Prior to use, an aliquot from the biotin-Aβ(1-42) stock (1 mg/mL in HFIP) was diluted 20× in HFIP, and HFIP was blown off by a stream of pure nitrogen gas from the glass vial containing biotin-Aβ(1-42). It was redissolved in the same volume of trifluoroacetic acid (TFA) and incubated at room temperature for 10 min before drying with nitrogen. This was repeated before resolubilizing biotin-Aβ(1-42) with an aliquot of DMSO at a final concentration of ~4 μM.

Single-Site Biotin−Avidin Aβ(1-42) Oligomer Assay. The assay was modified from a previously published protocol.[10] Briefly, the capturing agent, NeutrAvidin (Pierce, Rockford, IL), was dissolved in sodium phosphate buffer (10 mM NaPi, pH 7.5) at 1 mg/mL as a stock solution and stored at −20 °C until use. To immobilize NeutrAvidin on the surface of a 96-well flat-bottom MaxiSorp immunomicroplate (VWR Int., Mississauga, ON, Canada), the stock NeutrAvidin was first diluted to 1000× with 10 mM NaPi, and wells were coated overnight at 4 °C. To reduce nonspecific binding, 200 μL of PBS (137 mM NaCl, 2.7 mM KCl, 5.4 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) with 0.02% sodium azide and 2 mg/mL fatty-acid free BSA/fraction V (BioShop, Burlington, ON, Canada) was added to each well and shaken for up to 2 h at room temperature before

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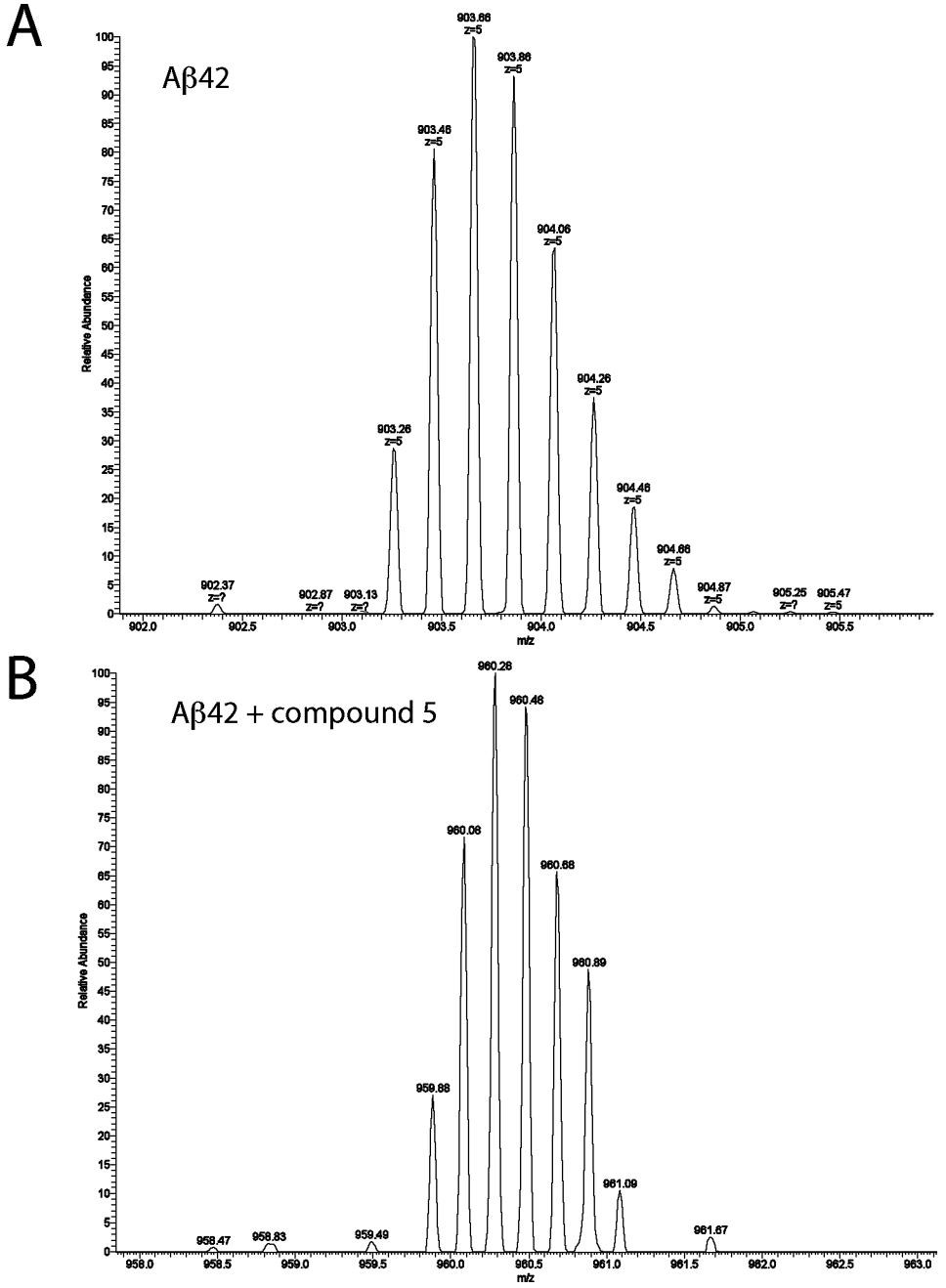

Figure 8. Mass Spectrometry analyses of *scyllo*-inositol azide and napthyl-derivatives. Monomeric Aβ1-42 was infused either alone (A) or mixed with compound **5** (B) at molar ratio of 1:10 in 100 mM ammonium bicarbonate buffer (pH 7.0) and subsequently infused directly into a hybrid LTQ Orbitrap mass spectrometer.

washing three times with TBST (20 mM Tris HCl, 140 mM NaCl, pH 7.3, with 0.1% TWEEN 20). Premixed incubation mixtures containing 15 nM disaggregated biotin-Aβ(1-42) with the appropriate molar ratio of compounds (1:5000, 1:500, 1:50, 1:5, and 1:0.5.) were placed on a shaker in a temperature-controlled incubator at 37 °C for 3 days. After 3 days, the plates were washed again with TBST. The detecting agent 2 μg/mL of streptavidin−horseradish peroxidase (SA-HRP) in TBST with 2 mg/mL of BSA was added to each well and incubated at room temperature for no more than 30 min before washing three times with TBST. Then 100 μL of 3,3′ 5,5′-tetramethylbenzidine (TMB) in liquid format for ELISA application (BioShop, Burlington, ON, Canada) was added to each well, and immediately placed in a VersaMax tunable microplate reader (Molecular Devices, Sunnyvale, CA). Optical density (OD) at 650 nm was monitored and recorded at 1 min intervals for 20 min. To compare between plates, the raw signals of each plate were normalized to the signal of the biotin-Aβ(1-42) alone signal. One-way ANOVA (factorial) and Bonferonni's posthoc tests for significance were performed using the statistical software package, STATVIEW.

Atomic Force Microscopy. To visualize the effect of screened compounds on Aβ(1-42) fibril assembly, 1 μL of stocked Aβ(1-42) without biotinylation (10 mg/mL, 2.2 mM in DMSO) was added to 50 μL of deionized water with appropriate amount of compound. After 14 days of incubation with shaking at 37 °C, 25 μL of each sample was deposited on a 0.5 in. diameter freshly cleaved muscovite mica surface and allowed to stand for 3 min before being dried with flowing nitrogen. Tapping-mode AFM imaging in air was performed under constant-amplitude feedback with TESP tips (manufacturer's specification: length = 110−140 μm, k = 20−80 N/m; Veeco Instruments, Woodbury, NY) using a Digital Instruments Multimode scanning probe microscope equipped with a Nanoscope IIIa controller, and a J-scanner with a maximum possible scan size of 90 μm × 90 μm.

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All images were acquired as 512 × 512 pixel data sets at a drive frequency between 270 and 300 kHz depending on the resonance frequency of individual probe cantilever used and a scan rate between 0.5 and 2 Hz. All images were processed with a zero-order flatten filter and a first-order plane fit using the Nanoscope software version 5.12 A. At least three different locations were screened for each sample and a representative image shown.

Aβ-Induced Toxicity. SH-SY5Y cells (ATCC, Manassas, VA) were seeded at a density of 50 000 cells/well in poly-L-lysine-coated 96-well plates and allowed to grow for 2 days prior to treatment. Aβ(1-42) (25 μM) and *scyllo*-inositol derivatives were incubated in the absence of cells for 1 day at 37°C with shaking. Aβ(1-42)-compounds were then incubated with the cells for 48 h. To determine the viability of the cells post-treatment, 0.5 mg/mL methylthiazolyldiphenyl-tetrazolium bromide (MTT) was added to the cultures and incubated for 30 min at 37 °C. The insoluble formazan dye formed in viable cells was solubilized by 100 μL dimethyl sulfoxide (DMSO). Absorbance of the solubilized dye was read on a Spectramax tunable plate reader (Molecular Devices) at 540 nm.

Mass Spectrometry. To determine the interaction between Aß1-42 and various *scyllo*-inositol derivatives, each compound was mixed with 1 mM Aß1-42 at molar ratio of 1:10 in 100 mM ammonium bicarbonate buffer (pH 7.0). The mixtures were then infused directly into a hybrid Orbitrap mass spectrometer through a nanospray ionization source containing an uncoated 10 μm i.d. SilicaTip PicoTip nanospray emitter (New Objective) and was operated in positive ion mode. The spray voltage was 1.8 kV, and the heated capillary temperature was 160−180 °C. MS1 data were acquired in the profile mode in the Orbitrap with five microscans and 100 ms injection time. The instrument was calibrated at the resolution of 60 000 at 400 *m*/*z*.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Complete ^{1}H and ^{13}C spectra for numbered compounds. Two figures to define the oligomerization assay. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(J.M.) Mailing address: Department of Laboratory Medicine and Pathobiology University of Toronto 1 King's College Circle Toronto, ON, CA M5S 1A2. E-mail: j.mclaurin@utoronto.ca. Telephone: 416-978-3554. Fax: 416-978-5959. (M.N.) Mailing address: Department of Chemistry, University of Toronto 80 St. George Street Toronto, ON, CA, M5S 3H6. E-mail: mnitz@chem.utoronto.ca. Telephone: 416-946-0640. Fax: 416-978-8775.

Author Contributions

⊥These authors contributed equally to the manuscript.

Author Contributions

J.E.S., A.Y.L., G.C.H.M., F.P., and L.A.M.T. performed the experimental work and data analysis. J.C. and S.D. performed the chemical synthesis and characterization. J.E.S., M.N., J.M., A.Y., and C.M.Y. performed the experimental plan, guidance, and manuscript writing and preparation.

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Notes

The authors declare the following competing financial interest(s):JM is named inventor on patents and patent applications relating to scyllo-inositol and derivatives. None of the other authors have competing interests.

■ ABBREVIATIONS

Aβ, amyloid-beta peptide; ThT, thioflavin T; ELISA, enzyme linked immunosorbent assay; AFM, atomic force microscopy; EGCG, epigallocatechin gallate; UV, ultraviolet; RP-HPLC, reverse phase high performance liquid chromatography; NMR, nuclear magnetic resonance; HFIP, hexafluoroisopropanol; Fmoc, 9H-(f)luoren-9-yl(m)eth(o)xy(c)arbonyl; DMSO, dimethyl sulfoxide; TBST, tris buffered saline tween; PBS, phosphate buffered saline; SA-HRP, streptavidin-horse radish peroxidase; TMB, 3,3′ 5,5′-tetramethylbenzidine; OD, optical density; MTT, methylthiazolyldiphenyl-tetrazolium bromide; *m*/*z*, mass charge ratio; ESI, electron spray ionization

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