Divergent Synthesis of scyllo-Inositol Aldoxime Derivatives as Potential Inhibitors of Amyloid-Beta(1-42) Aggregate Formation

by

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A thesis submitted in conformity with the requirements for the degree of Master of Sciences
Graduate Department of Chemistry
University of Toronto

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Abstract

scyllo-Inositol is currently in phase II clinical trials as a therapeutic for Alzheimer’s disease (AD). Previous work from our lab has shown that scyllo-inositol prevents Aβ_{1-42} fibril formation instead leading to the formation of small Aβ oligomers \textit{in vitro}. To further understand the molecular details of Aβ-scyllo-inositol binding interactions, a library of scyllo-inositol derivatives was prepared. A sequence of protecting group transformations afforded a hydroxylamine functionalized scyllo-inositol. Subsequent oxime formation with aromatic aldehydes generated a novel class of inositol derivatives in good yield and high purity. The effects of these compounds on the Aβ aggregation cascade were evaluated by a biotin-avidin Aβ_{1-42} oligomer assay and atomic force microscopy (AFM). Preliminary plate assay data indicated that several of these derivatives increased peptide oligomerization and the corresponding AFM images showed altered fibril formation. These results suggested that this class of scyllo-inositol derivatives is active in the Aβ aggregation cascade.
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List of Abbreviations

Aβ  amyloid-beta
AD  Alzheimer’s disease
APP β-amyloid precursor protein
aq  aqueous
Bn  benzyl
Boc tert-Butyloxy carbamate
Bz  benzoyl
°C  degree Celsius
DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
DCM dichloromethane
dH$_2$O de-ionized water
DIAD diisopropyl azodicarboxylate
DIPEA diisopropylethylamine
DMF N,N-dimethylformamide
DMSO dimethylsulfoxide
EGCG (-)-epigallocatechin-3-gallate
eq  equivalence
ESI-HRMS electrospray ionization high resolution mass spectrometry
Et  ethyl
Et₂O  diethyl ether
EtOAc  ethyl acetate
¹H/¹³C NMR  proton/carbon nuclear magnetic resonance
h  hour(s)
HPLC  high performance liquid chromatography
J  coupling constant
m/z  mass per charge
Me  methyl
NMDA  N-methyl-D-aspartate
NMP  N-methylpiperazine
PET  positron emission tomography
PhtN-NH₂  N-aminophthalimide
PhtN-OH  N-hydroxyphthalimide
PPh₃  triphenylphosine
ppm  parts per million
rt  room temperature
s  second
SA-HRP  steptavidin-horseradish peroxidase
SDS  sodium dodecyl sulfate
SMEAH  sodium bis(2-methoxyethoxy)aluminum hydride
<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>Tf</td>
<td>triflate/trifluoromethanesulphonyl</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>TFE</td>
<td>trifluoroethanol</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMB</td>
<td>3,3',5,5'-tetramethylbenzidine</td>
</tr>
<tr>
<td>Tris</td>
<td>tris(hydroxymethyl)aminomethane</td>
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1 Alzheimer’s disease

1.1 Neuropathy

Alzheimer’s disease (AD), a neurodegenerative disease, is the most common form of dementia in individuals over the age of 65. At early onset of AD, its clinical symptoms include deficits in memory and a series of mild cognitive impairments. Later progression entails global amnesia, deterioration of motor skills, and often mortality within nine years of diagnosis.\(^1\) Currently, half a million people in Canada are diagnosed with AD or related dementia. This figure is expected to reach 1.1 million within the next 25 years. As with any diseases the burden is twofold: personal challenges and lifestyle adjustments, as well as major socio-economic impact. In 2008, dementia had cost Canadians $15 billion. This figure is projected to rise to $153 billion by 2038.\(^2\) With a rapidly aging population in the developed world, Alzheimer’s disease is considered to be one of the greatest health challenges of the century. Therefore, continuous research is necessary to better understand the underlying biochemical mechanisms of Alzheimer’s disease to lead to therapeutic discovery.

Cerebral extracellular amyloid-beta (A\(\beta\)) plaques are one of the histopathological hallmarks of AD that was first described by German physician Alois Alzheimer in 1907.\(^3\) These A\(\beta\) plaques, mainly consisting of A\(\beta_{1-40}\) and A\(\beta_{1-42}\), begin to develop 10-30 years before clinical detection of the disease. These misfolded peptides self-assembles into higher order \(\beta\)-structures where the polypeptide is perpendicular to the fibril axis.\(^4\) A\(\beta_{1-42}\) has a higher propensity to form aggregates due to two additional hydrophobic residues (Ile41 and Ala42) at the C-terminus.\(^5\) The deposition of A\(\beta\) plaques commence at the neocortex of the human brain and proliferate hierarchically over time.\(^6,\,7\) A wealth of data from transgenic mice models of AD and human subjects support the notion that A\(\beta\) aggregation and accumulation results in neurodegeneration.\(^8-10\) In particular, soluble A\(\beta\) oligomers were found to be the primary neurotoxic form while A\(\beta\) fibrils may even be neuroprotective.\(^11-13\) Despite the immense amount of literature on AD, currently there is no cure for this disease and its etiology is still not well understood.
1.2 Amyloid-beta aggregation cascade

Aβ peptide is generated by proteolytic processing of the membrane-spanning β-amyloid precursor protein (APP) (Figure 1.1). β-secretase, a transmembrane aspartyl protease, produces the N-terminus of Aβ which is the rate-limiting step for liberation of Aβ from the APP. γ-secretase is a multisubunit aspartyl protease that cleaves APP in its transmembrane domain. The active complex requires presenilin 1 or 2 (catalytic core) and three accessory proteins: Aph1, Nicastrin, and Pen-2. γ-secretase is also responsible for processing other proteins such as Notch1, which has a critical role in cell growth, differentiation, and proliferation. γ-secretase catalyzes the C-terminal proteolysis step on APP. There have been efforts to target these two enzymes with inhibitors as a strategy to reduce Aβ production, however the accompanying adverse side effects found in mice models and humans have prevented their therapeutic use.

![Diagram](image)

**Figure 1.1** Aβ peptide production. Blocking a specific γ-secretase (containing Aph1B or Aph1A) might allow treatment of AD or cancer without altering the normal function of other γ-secretases.

Monomeric Aβ peptides do not aggregate into insoluble fibrils through a linear pathway (Figure 1.2). Distinct intermediates have been identified which either go on to form fibrils, termed on-pathway intermediates, or do not, termed off-pathway intermediates. The assembly of all Aβ species is an equilibrium process characterized by size rather than the number of Aβ peptides.
Purified Aβ1-40 and Aβ1-42 peptides after solid-phase peptide synthesis are soluble random-coils as confirmed by circular dichroism spectroscopy (Figure 1.2). Structural transition to a misfolded β-sheet intermediate is followed by recruitment of Aβ monomers to form an ordered nucleus. This transformation is rate limiting to subsequent nucleation-dependent aggregation. Once the critical protein concentration is reached, thermodynamically favourable growth of oligomers and fibrils occurs. These metastable aggregation intermediates including dimers and trimers are stable in detergents such as SDS. The addition of Aβ monomers to the oligomer nucleus results in protofibril production, which are short, β-sheet rich, rod-like structures. Recent structural studies revealed that two protofibrils wind around a hollow core to form a fibril strand. Although Aβ fibrils are thermodynamically stable compared to the other on-pathway intermediates, these fibrils exist kinetically in equilibrium with all species. As a result, an indirect approach to Aβ fibril disassembly is the use of Aβ binding partners and Aβ aggregation inhibitors to decrease the concentration of one or more species along the aggregation cascade.
1.3 Current treatments

There are five medications approved by the FDA that are designed to control the symptoms of AD and improve functioning. Three of these treatments are acetylcholinesterase inhibitors [Razadyne® (galantamine), Exelon® (rivastigmine), Aricept® (donepezil), Cognex® (tacrine)]. Inhibition of acetylcholine breakdown in the brain increases its bioavailability for intercellular communication and slows the progression of cognitive impairment. The fifth drug is an N-methyl-D-aspartate (NMDA) antagonist [Namenda® (memantine)]. Memantine modulates neuronal glutamate activity, reduces cellular calcium influx and cell degeneration. Although these medical treatments help ameliorate the symptoms, there is still an acute need to find therapeutics to directly treat this debilitating disease.

1.4 Small molecule inhibitors of Aβ1-42 fibril formation

Polyphenols have been found to possess anti-aggregation properties, as well as secondary effects including anti-oxidant and anti-inflammatory properties (Figure 1.3).

![Chemical structures]

**Figure 1.3** Anti-aggregant molecules.

(-)-Epigallocatechin-3-gallate (EGCG) is a member of a family of plant-derived flavan-3-ols, and is found in green tea. EGCG directly binds to native unfolded Aβ and promotes the formation of spherical oligomers which are unable to seed fibrillogenesis. Importantly, EGCG-stabilized oligomers are non-toxic to cells and in vivo administration of EGCG to Tg2576 mice decreased Aβ plaque load by as much as 50%.
Curcumin is the main component of the spice turmeric, and is speculated to be responsible for the considerably lower prevalence of AD in the Indian population.\textsuperscript{34} Curcumin has been found to inhibit Aβ\textsubscript{1-42} oligomerization, fibrillogensis, and Aβ-induced toxicity \textit{in vitro}, as well as bind to plaques and reduce Aβ levels \textit{in vivo}.\textsuperscript{35,36} There are three structural features of curcumin that are important for binding to Aβ and inhibit Aβ aggregation: a hydroxyphenyl moiety, a rigid linker region between 8 and 16 Å in length, and a second phenyl group.\textsuperscript{37} Similarly, polyphenolic grape seed extracts, including resveratrol, block Aβ fibril formation by preventing β-sheet structural transition, therefore decreasing oligomer and protofibril levels.\textsuperscript{38}
2  scyllo-Inositol

2.1  Introduction

Inositol s are essential constituents for biological function and necessary dietary component for all vertebrates. Inositols are cyclohexanehexols with one hydroxyl per carbon atom. The various stereochemistries give rise to isomers with different biochemical properties and physiological roles (Figure 2.1). myo-Inositol, the most common isomer, is the core unit of phosphatidylinositol in phospholipid membranes and facilitates secondary messenger signal transduction. Over the past 20 years several of the inositol isomers have been successfully evaluated as therapeutics for assorted ailments. myo-Inositol has been administered to treat psychiatric disorders such as depression and panic disorders. In mice it has been shown that epi-inositol can alleviate anxiety and D-chiro-inositol can prevent folate-resistant mouse neural tube defect. In humans, decreased levels of D-chiro-inositol have been correlated with insulin resistant type 2 diabetes and polycystic ovary syndrome.

scyllo-Inositol is a symmetric inositol isomer with all the alcohols oriented equatorially. The human brain maintains approximately 5 mM myo-inositol and 0.5 mM scyllo-inositol concentrations, while both conformers enter the brain either via active transporters or de novo synthesis from glucose metabolism. These inositols also act as osmolytes to maintain homeostasis. Altered cerebral metabolism of both myo- and scyllo-inositol and deviations from their physiological levels were found in diseased states such as chronic alcoholism, hepatic encephalopathy, and prostate cancer.

Figure 2.1 Stereoisomers of inositols.
In 1998, McLaurin et al. discovered that myo-inositol, the head group of phosphatidylinositol, inhibited Aβ1-42 fibrillogenesis.\textsuperscript{50} Further studies lead to the finding of scyllo-inositol which prevented Aβ1-42 fibril formation leading to stabilized small oligomers \textit{in vitro}.\textsuperscript{51} This stabilization of scyllo-inositol-Aβ complex rescued NGF-differentiated PC-12 cells from Aβ1-42 induced cytotoxicity. In a TgCRND8 transgenic mouse model of AD, administration of scyllo-inositol lead to decreased Aβ1-40 and Aβ1-42 plaque levels, synaptic dysfunction, reversed cerebral Aβ pathology, and reduced mortality rate observed in untreated mice.\textsuperscript{52} The Aβ disassembly and \textit{in vivo} therapeutic attributes appeared to be stereospecific, as myo- and epi- isomers were less effective. As a result, scyllo-inositol is currently in phase II clinical trials as an AD therapeutic.\textsuperscript{53} Despite these promising studies little is known about how scyllo-inositol interacts with Aβ1-42.

Previous work in our laboratory in collaboration with the McLaurin research group investigated the interactions between scyllo-inositol and the Aβ1-42 peptide. A series of scyllo-inositol derivatives were synthesized to provide information about hydrogen bonding requirements, hydrophobicity, and steric interactions of a given hydroxyl group in the scyllo-inositol-Aβ-peptide interaction (Figure 2.2).\textsuperscript{54} Of the eight derivatives evaluated by electron microscopy, the 1-deoxy-1-fluoro and 1,4-dimethoxy analogues were found to inhibit Aβ fibril growth similarly to the parent compound (Figure 2.3). In all other cases, minor modifications of one or both hydroxyl groups in the 1,4- positions resulted in inactivity of the compounds, which implicated that polar atoms around the inositol skeleton were important for preserving scyllo-pharmacophore.

![Figure 2.2](image-url) Previouly synthesized mono- and di-substituted scyllo-inositol derivatives.
Given the activity of 1-deoxy-1-fluoro-scyllo-inositol it was hypothesized that this scaffold may serve as a promising PET imaging agent for AD. The fluorine-18 labelled scyllo-inositol derivative was synthesized in collaboration with the Vasdev laboratory and evaluated as a PET imaging agent (Figure 2.4). The radiotracer was observed to have low brain penetration in a CD1 nude mouse model over the short lifetime of the radionuclide due to its low lipophilicity. In later mouse dosing studies it was found significant brain penetration of 1-deoxy-1-fluoro-scyllo-inositol could only be achieved through chronic dosing. Despite this disappointing result as an amyloid imaging agent, the $^{18}$F-labelled compound observed to be retained in the MDA-MB-231 breast cancer cell line and is currently being investigated in cancer imaging studies.
**Figure 2.4** 2 h dynamic small animal imaging studies. Coronal images of fasted female CD1 nude mice post injection of (a) $[^{18}\text{F}]-1$-deoxy-1-fluoro-scyllo-inositol. (b) Image through the plane of the brain at 3 min. (c) Image through the plane of tumour at 45 min (peak), 14 days post-inoculation of MDA-MB-231 tumour cell line in right shoulder.
2.2 Scope and aims

Our previous investigation on Aβ1-42-scyllo-inositol structure-activity relationships has indicated the importance of conserving all polar hydroxyl groups along the inositol framework to retain pharmacophore. Removal of one hydroxyl and replacement with a hydrogen atom rendered the compound inactive. To further our understanding of the molecular dynamics between Aβ-scyllo-inositol interactions, we considered scyllo-inositol derivatives that maintained the key heavy atoms. We explored the hydroxylamine functionality because in this derivative only a hydrogen atom is substituted for a nitrogen atom. In addition, the hydroxylamine allowed direct functionalization via oxime ligation with various aldehydes to produce a library of scyllo-inositol derivatives with moderately different chemical and physical properties. More specifically, phenolic aldehydes were of particular interest as many compounds that inhibit Aβ fibrillization contain phenol groups. Currently, there have not been literature reports on the synthesis of this class of inositol derivatives. This Master’s project looked to establish the chemistry to 1) transform myo-inositol to 1-O-amino-scyllo-inositol through a series of strategic protecting group manipulations, and 2) rapidly acquire a library of scyllo-inositol derivatives through chemoselective oxime conjugation with various substituted aromatic aldehydes. Although this thesis reports oxime ligation with aldehydes, ketone derivatives may also be considered. Similarly, this chemistry can be extended to the analogous preparation of hydrazine and hydrazone library. To investigate the significance of the spatial orientation of the aryl moiety relative to the inositol core, three other types of linkages were evaluated: amide, carbamate, and hydroxamate. Somnath Dasgupta, a postdoctoral fellow in our laboratory, synthesized the amide and carbamate linked scyllo-inositol derivatives. This thesis reports the synthesis of two hydroxamate derivatives.

The Aβ-scyllo-inositol interactions were tested by our research collaborators. James Shaw, from the McLaurin research group, developed a biotin-avidin Ab1-42 oligomer assay to measure changes in peptide oligomerization. In addition, inositol-induced modulations to Aβ1-42 fibrillation were confirmed with atomic force microscopy (AFM). Preliminary results from the two assays are discussed in this Master’s thesis.
2.3 Chemoselective oxime ligation

Oxime formation features condensation of a free amine with an electrophilic aldehyde or ketone (Scheme 2.1). Oximes are more stable towards hydrolysis than imines due to the participation of the oxygen atom in the electron delocalization. This transformation is orthogonal to many organic functional groups and high yielding, which enables the rapid synthesis of a large library of compounds that could be optimized for automation. This approach has been used to generate a library against human small cell lung carcinoma (A549) cells and anti-inflammatory agents.

\[
R_1\text{O}^\text{NH}_2 + \text{HOR}_2 \xrightarrow{\text{pH 4-5}} \xrightarrow{-\text{H}_2\text{O}} \ R_1\text{ON}^\text{H}_2 \text{R}_2
\]

Scheme 2.1 Oxime formation.

One example of oxime used by nature is in the biosynthesis of dhurrin (Scheme 2.2). There have been numerous reports of biologically active oxime ether containing molecules, several of which have inhibitory properties towards Aβ fibrils. Also there are FDA approved drugs with the oxime moiety, suggesting this functional group is metabolically stable.

Scheme 2.2 The E/Z-isomers of p-hydroxyphenylacetaldehyde oxime as intermediates in the biosynthesis of dhurrin in Sorghum bicolor (L.) Moench.
Numerous synthetic approaches of hydroxylamines have been reviewed. For example, installation of the hydroxylamine moiety is achieved via alkylation, arylation, reduction, or oxidation (Scheme 2.3).

Scheme 2.3 Examples of hydroxylamine synthesis.
3  **scyllo-Inositol derivatives**

3.1  **1-O-Amino-scyllo-inositol hydrochloride**

Synthesis of the hydroxylamine precursor required strategic protecting group modifications to obtain the scyllo- configuration (Scheme 3.1). Intermediates 2-4 were synthesized following published procedures previously developed in our lab.\textsuperscript{54} myo-Inositol 1 was chosen as the starting isomer since the compound is commercially available and inexpensive.

**Scheme 3.1** Synthesis of 1-O-amino-scyllo-inositol hydrochloride. (a) 2,3-butanedione, p-TSA, CH(OMe)\textsubscript{3}, MeOH, 2, 27%; (b) BzCl, CH\textsubscript{2}Cl\textsubscript{2}/pyridine (1:1), 3, 65%; (c) Tf\textsubscript{2}O, CH\textsubscript{2}Cl\textsubscript{2}/pyridine (17:1), 4, 70%; (d) phtN-OH, Et\textsubscript{3}N, DMPU, 5, 76%; (e) 95% TFA, 6, 98%; (f) i) MeNH\textsubscript{2}/MeOH, ii) conc. HCl, 7, 59%.
In a one step process, two sets of trans-diols were acetal protected with 2,3-butanedione to give the diol intermediate 2 in 27% yield. Although low yielding this reaction can readily be carried out at scale as only the product crystallizes from the crude reaction mixture. The equatorial hydroxyl was acylated with benzoyl chloride selectively over the axial position in 65% yield; however di-substitution could not be avoided and commonly 10% was isolated in this reaction. The crude mixture of mono- and di-benzoylated compounds exhibited poor solubility in many organic solvents and therefore column chromatography on a large scale was problematic. The following step installed a triflate leaving group at the axial hydroxyl group to afford myo-trflate 4 in 70% yield.

A number of different approaches were attempted to introduce the hydroxylamino substituent with inversion of the axial alcohol of myo-inositol to give the scyllo-configuration. The desired hydroxyamino group could be derived from PhthN-OH (N-hydroxphthalimide) or N-Boc-hydroxylamine. Initial attempts using S_N2 displacement of the triflate group with PhthN-OH or N-Boc-hydroxylamine in DMF and base resulted in elimination and a minimal or none of the desired product (Scheme 3.1, bottom). Using Mitsunobu conditions with mono-benzoyl 3 did not afford the phthalimido 5, despite varying the equivalence of reagents, temperature, or solvents (Scheme 3.1, middle). The lack of reactivity can be explained by steric hinderance of the acetal groups in proximity to the cyclic secondary carbon center. The β-elimination and lack of Mitsunobu reactivity was also observed by Xie et al. with a D-glucofuranose derivative when using PhthN-OH as the nucleophile. On the contrary, Cheng et al. reported successful inversion of 2-OH of a less sterically hindered myo-inositol derivative under Mitsunobu conditions using benzoic acid as a nuclepheophile (Scheme 3.2).

Scheme 3.2 Inversion of myo-configuration to scyllo-configuration via Mitsunobu reaction.

Vasella et al. reported conversion of a perbenzylated galacto-configured triflate to phthalimido-glucose derivative in DMPU (1,3-dimethyl-3,4,5,6-tetrahydropyrimidin-2(1H)-one). The conditions were applied to triflate 4 and observation of the crude product by TLC and ^1H NMR
spectroscopy indicated that phthalimide 5 was formed along with 10-15% elimination product. DMPU has a high boiling point such that evaporation under reduced pressure is not feasible. Organic extraction using EtOAc, Et₂O, or DCM to remove DMPU proved ineffective. Phthalimide 5 was not recovered in EtOAc or Et₂O, while the third case saw DMPU preferentially partitioned into DCM. Extraction of the solvent using concentrated aqueous salt (DCM and 1.0 M LiCl) was also unsuccessful. Alternatively, addition of water (10:1 H₂O:DMPU) to the reaction mixture caused the product 5 to precipitate. The crude precipitation was cooled to 4 °C and filtration recovered about 80-90% of the desired product. Further purification by silica gel chromatography gave phthalimide 5 in 76% yield. The acetal protecting groups of compound 5 were readily removed with 95% aq. TFA to afford intermediate 6 in 98% yield. Aminolysis with MeNH₂ proceeded smoothly removing both benzoate and phthalimido protecting groups. This reaction progressed more cleanly than similar attempts using hydrazine. The highly polar character of 1-O-amino-scylllo-inositol, in addition to similar solubility as the by-products, made the purification problematic. Conventional separation techniques such as liquid-liquid extraction and C-18 solid phase extraction were ineffective, while silica gel chromatography was not a viable option due to the polarity of the compound. Recrystallization of the crude mixture in ethanol gave low yields because of the stoichiometric by-products resulting from the protecting groups and furthermore, N-methylphthalimide and methylbenzamide proved to be highly crystalline. It was found that hydroxylamine 7 as a hydrochloride salt exhibited lower solubility in absolute ethanol than the by-products, allowing product 7 to be isolated in 59% yield.
3.2 Arylaldoximes

Modified experimental conditions for oxime formation were adopted from Ulf Nilsson’s work on the synthesis of \(O\)-galactosyl aldoximes as galectin-3 inhibitors.\(^{72}\) In all instances, synthesis of oximes 8-11 proceeded at room temperature by simply mixing the hydroxylamine hydrochloride salt with the desired aldehyde (Scheme 3.3-3.5). Most of the reactions went to completion after several hours, but were stirred overnight to ensure that no starting hydroxylamine remained. It was found that high concentration of hydroxylamine \(7\) (0.04-0.06 M) was required to drive the equilibrium. Reaction with benzaldehyde to give oxime 8 was completed after 10 minutes in THF:H\(_2\)O (1:1) and was purified by organic extraction with Et\(_2\)O or EtOAc (Scheme 3.3). Compound 8 was isolated in 90% yield as the \(E\)-isomer. \(Di\)-hydroxyls 9-11 were stirred in MeOH:H\(_2\)O (2:1) in which the products precipitated out of solution as the reaction progressed. Organic extraction and C-18 SPE work-up were necessary to remove excess aldehydes. Differences in water solubility were observed for the three constitutional isomers: compound 9 was readily soluble in water and compound 11 was poorly soluble, while compound 10 was partially soluble. \(Di\)-hydroxyl derivatives 9-11 were obtained in 78% as the \(E\)-isomer only, likely due to hydrogen bonding between the \(ortho\)-hydroxyl and the oxime nitrogen.

\[ \text{Scheme 3.3 Synthesis of arylaldoximes 8-11.} \]
RP-HPLC was found to be the best method to purify oximes 12-19 compared to liquid-liquid extraction and C-18 SPE due to their poor solubility in water. Oximes 12-14 were prepared in THF:H₂O (1:1) because 1-naphthaldehyde and 2-furaldehyde were miscible in this mixture and not in MeOH:H₂O (Scheme 3.4). Compounds 12-14 and were obtained after lyophilization in 70, 72, and 39% yields, respectively. Naphthyl 12 and quinolyl 13 were in the E-isomeric form exclusively. Furfuryl 14 was isolated in 2:1 E:Z mixture, as determined by ¹H NMR spectroscopy integrations. The low yield of 39% was an artifact of the HPLC purification step and not due to incomplete reaction.

![Scheme 3.4 Synthesis of arylaldoximes 12-14.](image)

Phenyl oximes 15-19 were conveniently prepared as the products formed precipitate in all cases except di-hydroxyl 18 (Scheme 3.5). Mono-substituted aryloximes 15-17 were obtained only as the E-isomer in 71, 72, 64% yields, respectively. 3,4-Dihydroxyl 18 was found to decompose after organic extraction and C-18 SPE purifications. RP-HPLC afforded product 18 as a 3:1 E:Z mixture in 72% yield. However, compound 18 was found to be unstable, decomposing to a black solid on standing at room temperature for a month either through oxime hydrolysis or oxidation by atmospheric O₂. This observation suggests that an ortho- hydroxyl group increases the compound stability through hydrogen bonding with the oxime nitrogen. Tri-hydroxylated 19 was obtained in 73% yield in the E-isomeric form only.
Scheme 3.5 Synthesis of arylaldoximes 15-19.
3.3 Hydroxamate derivatives

Acylation of hydroxylamine 7 gave access to hydroxamate derivatives with potentially different biochemical properties than the oxime library (Scheme 3.6). The phthalimido group of 5 could be selectively removed with MeNH₂ while the benzoyl ester remains intact. This selectivity arose due to insolubility of the protected hydroxylamine 20 which precipitated out of the reaction after phthalimido aminolysis. Intermediate 20 was afforded in 86% yield. This selectivity allows convenient derivation of the free hydroxylamine substituent without undesired reactions on the ring oxygen atoms. The use of 1.5 equivalents of BzCl resulted in the di-benzoylated intermediate 21 in 74% yield. Transesterification with sodium methoxide in methanol removed the second N-benzoyl as well as the O-linked benzoyl to afford mono-hydroxamate 22, which was directly used in the next step. Acetal hydrolysis with 95% TFA and silica gel chromatography gave the final product 23 in 52% yield over two steps. The hydroxamate linkage was found to be stable under ambient conditions for over two months.

Scheme 3.6 Synthesis of 1-O-benzamido-scyllo-inositol. (a) 2 M MeNH₂ in MeOH, 20, 86%; (b) BzCl, Et₃N, CH₂Cl₂, 21, 74%; (c) NaOMe/MeOH; (d) 95% TFA, 23, 52% over two steps.
Initial Aβ₁₋₄₂ oligomer assay results and AFM analysis on oximes 8-11 suggested that a hydroxyl group in the para- position may be important for modulating Aβ₁₋₄₂ secondary structures. Therefore 4-hydroxyphenyl 27 was a target of interest (Scheme 3.7). Seeing that hydroxamate 21 was di-acylated in the previous scheme, hydroxyamine 20 was reacted with 2.1 eq. 4-(BnO)BzCl [4-(benzyloxy)benzoyl chloride], 2.1 eq. Et₃N, in anhydrous DCM at 0 °C for 17 h in order to obtain di-acylated 24b in greater than 75% yield. In this reaction, however, mono- 24a was found to be the major product.

Scheme 3.7 Synthetic route of 1-O-(4-hydroxy)benzamido-scyllo-inositol. (a) 4-(BnO)BzCl, Et₃N, CH₂Cl₂; (b) NaOMe/MeOH; (c) H₂, Pd/C; (d) 95% TFA.

The reaction was repeated using 1.2 eq. 4-(BzO)BzCl and 1.2 eq. Et₃N, but unexpectedly a mixture of mono- 24a and di- 24b (minor) was observed. Silica gel chromatography using 3:1 pentane:ethyl acetate, 5:1 pentane:ethyl acetate, or 1% MeOH in DCM eluent mixtures did not result in complete separation of 24a and 24b, while the third solvent choice gave the best results.
Excess 4-(benzyloxy)benzoic acid also eluted with the less polar 24b. The $^1$H NMR spectrum of mono- 24a in CDCl$_3$ at 25 °C contained broad peaks due to rotational restriction about the N-C=O amide bonds. The mixture 24a + 24b was carried forward for benzoyl cleavage with NaOMe/MeOH at room temperature for 48 h. The N-benzoyl group was cleaved first before the O-benzoyl ester. The reaction mixture was neutralized with Amberlite IR-120 H$^+$ exchange resin and purified by silica gel chromatography to obtain intermediate 25 in 54% yield over 2 steps. Standard hydrogenation conditions removed the benzyl protecting group to yield diol 26, however the proton spectrum of this intermediate contained unidentifiable impurities. Diol 26 was reacted with 95% TFA for acetal hydrolysis, followed by silica gel purification (15% MeOH in DCM). However, less than 10% of product 27 was isolated while the $^1$H NMR spectrum revealed the desired product along with 4-hydroxybenzoic acid. Although the target 4-hydroxyphenyl hydroxamate 27 was synthesized successfully, the isolation steps were challenging resulting in low yields and an impure product.
3.4 Hydrazine derivatives

The established chemistry for hydroxylamine synthesis was applied to the development of hydrazine derivatives. Several methods were attempted to introduce a hydrazine functional group to the inositol ring (Scheme 3.8). Nucleophilic substitution on myo-triflate 4 with hydrazine hydrate under reflux conditions returned starting material and the β-elimination product. Substitution with hydrazine proved to be unfavourable despite high temperature and long reaction times.

Scheme 3.8 Synthetic routes to hydrazine derivatives.
Substitution with PhtN-NH₂ (N-aminophthalimide) in DMPU gave compound 28 as the major product, some β-elimination by-product, and excess PhtN-NH₂. Crude 28 was precipitated out from DMPU by H₂O addition and collected by filtration. Unfortunately, TLC analysis showed compound 28 and Pht-NH₂ had an Rf difference of < 0.1 [pentane:ethyl acetate (3:1, 2:1, 1:1) or 4% MeOH in DCM]. Separation of the two compounds was unsuccessful by column chromatography. In hopes to increase product separation with increasing polar character, impure 28 was carried forward with two routes of protecting group removal. Crude 28 was reacted with 95% TFA for three hours, followed by evaporation of TFA and 2,3-butanedione with a continuous stream of N₂ gas. The proton NMR spectrum of 29 showed cleavage of the acetal groups and upfield shift of the inositol protons. The crude compound 29 was subsequently added 2 M MeNH₂ in anhydrous MeOH and stirred overnight at room temperature. The reaction mixture was concentrated to a brown solid and analysis by ¹H NMR spectroscopy of the residue in D₂O indicated that it was not the desired hydrazine derivative 32. Alternatively, crude phthalimide 28 was reacted with MeNH₂ at room temperature for 24 h and intermediate 30 was isolated as a white solid. Acid hydrolysis of the unpurified compound 30 with 95% TFA cleanly gave compound 31. The final deprotection step of O-benzoyl removal with NaOMe/MeOH did not afford hydrazine 32 after work-up with Amberlite H⁺ cation exchange resin, possibly because the charged inositol derivative was also taken up by the resins. Benzoyl ester deprotection using MeNH₂/MeOH was also unsuccessful, as the desired product was not recovered in the aqueous layer of DCM/H₂O extraction work-up. Despite not purifying each intermediate until the last step, the accumulation of side products also made isolation of the desired compound difficult. Although the hydrazine 32 was not successfully obtained, the efforts outlined in this thesis lay the ground work for future pursuit of the compound.
3.5 Bis-*scyllo*-inositols

Given the oligomeric nature of the Aβ-peptide that is stabilized by *scyllo*-inositol, it is reasonable to assume that multiple inositols are binding to each aggregate. Thus, we wished to explore the possibility of synthesizing inositol oligomers that may have greater avidity towards the Aβ-oligomers and have improved biological activity.

We examined the use of oxime ligation by means of an aromatic moiety possessing two aldehydes as an approach to tether two *scyllo*-inositol compounds. In 2003, Hagiya et al. described a simple and mild reduction of aromatic di-esters to di-aldehydes using SMEAH [sodium bis(2-methoxyethoxy)aluminum hydride]. This approach enabled access to many types of aromatic di-aldehydes from their commercially available and relatively cheap methyl-esters. Employing literature procedures, 2,6-naphthalene-dicarboxaldehyde was obtained cleanly in 69% yield and the proton NMR spectral data was confirmed (Scheme 3.9, top).

Reduction of dimethyl 1,3-benzenedicarboxylate occurred readily as observed by a considerable colour change from dark blue to bright yellow (Scheme 3.9, bottom). The di-aldehyde was challenging to purify and the final product contained 20-30% partially reduced mono-ester.

![Scheme 3.9 SMEAH reduction of methylesters to aldehydes.](image)

Attempts were taken to synthesize three different di-oximes (Scheme 3.10). Our expectation was that the bis-conjugation would proceed smoothly as it did in the preparation of *mono*-oxime
derivatives. Using 2.4 equivalence of hydroxylamine 7 and 2,6-naphthalene dicarboxaldehyde in DMSO, the reaction was completed after stirring overnight. The di-inosityl naphthalene product 33 was precipitated by addition of H2O and collected by vacuum filtration. When the reaction was repeated in different solvent conditions, a white precipitate was observed in THF:H2O (1:1), MeOH:DCM (10:1), TFE:H2O (1:1), DMF, and toluene:EtOH:H2O (1:1:1). Analysis of the crude product under these conditions by 1H NMR spectroscopy in D2O and TLC indicated formation of the mono-oxime product and incomplete reaction.

\[
\begin{align*}
\text{HO} & \quad \text{OH} & \quad \text{OH} & \quad \text{NH}_2 \cdot \text{HCl} & \quad \text{C}=\text{O} & \quad \text{Z} = \begin{array}{c}
\text{Z} \\
\text{H} \\
\text{H}
\end{array} \\
\text{7} & \quad \text{O} & \quad \text{O} & \quad \text{Z} & \quad \text{N} & \quad \text{Z} & \quad \text{N} & \quad \text{R}
\end{align*}
\]

**Scheme 3.10** Synthetic route to di-oximes.

The preparation of 1,3-benzene di-oxime was pursued using a high concentration of hydroxylamine 7 (2.4 eq, 63 mM) and impure 1,3-benzene dicarboxaldehyde from the SMEAH reduction in THF:H2O (1:1). The reaction was stirred overnight and purified by RP-HPLC, which isolated the methylester mono-oxime 34a and mono-scyllo-inosityl oxime 34b (Figure 3.1). Further studies are necessary to understand why only a single oxime was formed under these conditions.

\[
\begin{align*}
\text{HO} & \quad \text{OH} & \quad \text{OH} & \quad \text{O} \quad \text{OH} & \quad \text{O} \quad \text{HO} & \quad \text{O} \quad \text{OH} & \quad \text{OH} & \quad \text{O} & \quad \text{C}=\text{O} & \quad \text{Me} \\
\text{34a} & \quad \text{34b}
\end{align*}
\]

**Figure 3.1** Isolated oxime derivatives by RP-HPLC.
Similarly to the preparation of the naphthalene derivative, a solution of 1,4-benzene dicarboxaldehyde (Sigma Aldrich) and 2.4 equivalence of hydroxylamine 7 in DMSO was stirred overnight at room temperature. Water was added to precipitate the product and 1,4-phenyl-\textit{di}-oxime 35 was filtered and collected in 15-25\% yield. Proton NMR spectroscopy analysis of the product showed residual aldehyde, evidence that the reaction was not completed or hydrolysis occurred during the precipitation step. The reaction was also attempted using THF:H$_2$O (1:1) but was less successful compared to the first approach. The reaction progress under this condition was monitored by TLC which showed the emergence of two new spots, possibly \textit{mono}-oxime and \textit{di}-oxime products. Further additions of hydroxylamine 7 in THF:H$_2$O did not lead to a change in product distribution. As observed with the naphthalene aldehydes, the oxime products were found to be insoluble in a variety of common solvents. Although the synthesis of \textit{di}-oximes appeared attractive, their insolubility proved to make their synthesis experimentally challenging. Future work is needed to improve the isolation efficiency of 35 from DMSO by taking advantage of its insolubility in other common solvents.
4 $^1$H NMR peptide-ligand binding experiment

4.1 Study objective and methods

Proton NMR spectroscopy is a useful analytical tool for quantitatively determine interactions between a peptide and ligand. These interactions are quantified by two physical parameters: chemical shift of the NMR peaks and line-width broadening. The proportionally large A$_{\beta1-42}$ peptide oligomers would be expected to exhibit fast relaxation time and broad line-widths, while scyllo-inositol (MW 180) has a slow relaxation time and sharp NMR peaks. Upon peptide-ligand binding, alterations to the local environment immediately surrounding the ligand cause the ligand NMR peaks to shift and broaden.

The goal of this experiment was to determine whether the scyllo-inositol-A$_{\beta1-42}$ peptide binding interactions could be observed, as well as quantified, by $^1$H NMR spectroscopy. If scyllo-inositol binds to A$_{\beta1-42}$ aggregates on the NMR time-scale ($10^{-5}$ s), we would expect to observe a shift in NMR peak for the inositol protons and the corresponding peak broadening, as well as a decrease in proton integration for the $^1$H NMR peak of free scyllo-inositol. Validation of this experiment would allow competitive inhibitor studies and compound screening to be performed.

In the first trial experiment, scyllo-inositol (0.31 mM) was mixed with A$_{\beta1-42}$ peptide (0.31 mM) in 700 $\mu$L D$_2$O. Tris-HCl (0.31 mM) was added as an internal standard that would not bind to the A$_{\beta1-42}$ peptide. The proton integration of scyllo-inositol (6H’s) was monitored and compared to the proton integration of Tris (6H’s) to determine if scyllo-inositol-A$_{\beta1-42}$ oligomer binding had occurred. The spectra were taken at 300 MHz or 400 MHz with a 15 second relaxation time and 32 scans. Tris and scyllo-inositol appear at 3.75 and 3.36 ppm, respectively, relative to tetramethylsilane.
4.2 Results and discussion

Aβ_{1-42} peptide was prepared by solid-phase peptide synthesis and purified by RP-HPLC. Treatment of Aβ_{1-42} peptide with dilute ammonium hydroxide over three days resulted in structural transition from random coils to β-sheet (Figure 4.1). ^1^H NMR spectrum of the insoluble Aβ_{1-42} aggregates (1 mg) in D$_2$O (700 μL) at 25 °C showed a flat baseline (Figure 4.2). The Aβ_{1-42} sample was subsequently incubated with 1 equivalent of both scylo-inositol and Tris-HCl for a final concentration of 0.31 mM for each component. The sample was monitored by ^1^H NMR spectroscopy each day and analyzed for changes in chemical shift and peak broadening of scylo-inositol (3.36 ppm) and Tris-HCl (3.75 ppm).

![CD of Aβ_{1-42} after aq. NH$_4$OH and NaN$_3$ treatment](image)

**Figure 4.1** Circular dichroism spectrum of Aβ_{1-42} sample.

Unfortunately, no significant changes to the ^1^H NMR spectrum were observed from day 0 to day 7 under the investigated NMR parameters. On day 7, a 60-fold excess of scylo-inositol (2.4 mg, 19 mM final concentration in sample) was added to the NMR sample to determine whether any peak shifts would occur at higher ligand concentration. Surprisingly, we did not observe a change in chemical shift for scylo-inositol from day 7 to day 16, despite scylo-inositol is known to bind Aβ_{1-42} aggregates. Also, the proton integration of scylo-inositol remained unchanged relative to the integration of Tris-HCl (6H’s). A plausible explanation for the lack of binding
observed is that the Aβ1-42 aggregates were highly insoluble in D₂O and scyllo-inositol is unable to enter its Aβ1-42 aggregate binding sites. These initial spectroscopic data reinforced the complex nature and heterogeneity of Aβ1-42 aggregates, and indicated that ligand binding to Aβ1-42 aggregates may be dependent on the method of peptide preparation. Further evaluation of different NMR parameters and sample preparation methods is necessary to improve this ¹H NMR peptide-ligand binding experiment.

**Figure 4.2** Stacked ¹H NMR spectra of Aβ1-42 peptide treated with scyllo-inositol (3.36 ppm) and Tris-HCl (3.75 ppm) in D₂O at 25 °C.
5 Evaluation of arylaldoximes on Aβ1-42 aggregation

5.1 Biotin-avidin Aβ1-42 oligomer assay

To assess the biophysical properties of the synthesized scyllo-inositol derivatives on Aβ1-42 oligomerization, James Shaw of the McLaurin laboratory developed an Aβ1-42 oligomer assay based on the work described by Harry Levine.74 This assay utilized N-terminus labelled biotinylated-Aβ1-42 peptide to form soluble oligomers on a NeutrAvidin-coated plate, while SA-HRP (steptavidin-horseradish peroxidase) bioconjugate and TMB (3,3',5,5'-tetramethylbenzidine) substrate produced the corresponding fluorescence output. Hydroxylamine 7 and oximes 8-19 were evaluated as potential aggregate modulators. scyllo-Inositol and ECGC served as positive controls and d-chiro-inositol was tested as the negative control.

5.1.1 Assay principle

The principle of the assay is illustrated below (Figure 5.1). Biotinylated-Aβ1-42 peptide (10 nM) was incubated on a NeutrAvidin-coated plate in which the N-terminus of the Aβ1-42 peptides was bound to NeutrAvidin in a 1:1 ratio (n = 16). The plate was shaken for 3 days for oligomer formation. Oligomerization resulted in numerous exposed biotin sites of the Aβ1-42 peptides, therefore the biotin moiety was a marker for fluorescence detection. Subsequent treatment with SA-HRP bioconjugate and TMB substrate provided an optical readout at 650 nm.

![Figure 5.1 Principle of the mono-biotinylated Aβ1-42 oligomer screening assay.](image)
Consequently, the inhibitory activities of scyllo-inositol derivatives were evaluated by incubation with biotinylated-Aβ1-42 peptides at five ligand concentrations. These concentration-dependent fluorescence profiles for each compound represented characteristic modulatory activity on the Aβ1-42 oligomerization pathway. Ideally, an inhibitor of Aβ1-42 oligomerization should correlate to a decrease in fluorescence compared to Aβ1-42 control. Conversely, an increase in optical readout compared to control signifies an increase in oligomerization. The advantages of this assay include the capacity to evaluate many compounds at a time and more economical than using anti-bodies. The limitations include the fluorescence data reflect an indirect measurement of oligomerization and only small signal differences were observed even with known inhibitors or promoters of Aβ aggregation.

5.1.2 Preliminary fluorescence profiles of various scyllo-inositols

Preliminary results obtained from our collaborators indicated that several oxime derivatives actively modulated Aβ1-42 oligomer formation. In this assay, Aβ1-42 peptides (10 nM) were treated with compounds at five different concentrations (5 nM, 50 nM, 500 nM, 5 µM, 50 µM) (Figure 5.2). The positive control experiment with scyllo-inositol showed statistically significant increase in fluorescence at ligand concentrations of 500 nM and 5 µM. The increase in oligomerization was expected since scyllo-inositol is known to inhibit Aβ1-42 fibrillogenesis and form nontoxic small stabilized oligomers. This fluorescence profile also revealed that the promotion or inhibition of aggregation was concentration dependent with some compounds increasing oligomerization at low concentrations and preventing oligomerization at high concentrations. Previous assays that have only evaluated a single inhibitor concentration may have resulted in missing oligomerization active compounds. Treatment of Aβ1-42 with 50 µM phenyl oxime 8 showed a significant increase in oligomerization that was greater than scyllo-inositol. Incubation with 2,4-dihydroxyphenyl oxime 10 had a similar plate assay profile as oxime 8. The hydroxyl in the 4’ position was essential for altering oligomerization as relocation of the hydroxyl to either 3’ or 5’ positions (compounds 9 and 11) inactivated the compounds (data not shown). These initial data indicated that several scyllo-inositol derivatives were able to perturb the oligomerization pathway. Moreover, specific positioning of the aryl hydroxyl groups was demonstrated to be important for affecting Aβ aggregation cascade. Complete assessment
of the oxime library would provide a more conclusive understanding of the Aβ-scyllo-inositol structure-activity relationships.

Figure 5.2 Concentration dependent biotin-avidin Aβ1-42 oligomer assay profiles. Biotinylated-Aβ1-42 peptide (10 nM) was incubated with inositol treatment at 37 °C with shaking for 3 days to allow aggregate formation (n = 16). Treatment with steptavidin-horseradish peroxidase bioconjugate and 3,3',5,5'-tetramethylbenzidine (TMB) substrate produced optical readout at 650 nm. (a) scyllo-inositol; (b) compound 8; (c) compound 10. *P < 0.05 by ANOVA with Fisher PLSD.
5.2 AFM images

AFM (atomic force microscopy) was utilized to analyze scyllo-inositol induced modulation of Aβ1-42 fibril formation (Figure 5.3). Atomic force microscopy (AFM) images of Aβ1-42 (44 μM) were imaged after 14 days of incubation at 37 °C with treatment. (a) Aβ1-42 control; (b) scyllo-inositol (ligand:peptide = 500:1); (c) compound 8 (ligand:peptide = 10:1); (d) compound 10 (ligand:peptide = 10:1). Aβ1-42 (44 μM) was imaged after 14 days of incubation at 37 °C with or without treatment. Control Aβ1-42 peptide revealed long and fibrous aggregates (a). Fibril formation was inhibited upon incubation with scyllo-inositol (ligand:peptide = 500:1), as demonstrated previously (b). Instead, smaller globular aggregates were present. Phenyl-oxime 8 (ligand:peptide = 10:1) appeared to affect Aβ1-42 aggregation and formed clusters of small and threadlike aggregates (c). Incubation of Aβ1-42 peptides and 2,4-dihydroxy-oxime 10 (ligand:peptide = 10:1) reduced the formation of long fibrils as seen in Aβ1-42 alone (d). These preliminary AFM data suggest an active role of scyllo-inositol derivatives in the Aβ1-42 aggregation cascade.

Figure 5.3 Atomic force microscopy (AFM) images of Aβ1-42. Aβ1-42 (44 μM) was imaged after 14 days of incubation at 37 °C with treatment. (a) Aβ1-42 control; (b) scyllo-inositol
(ligand:peptide = 500:1); (c) compound 8 (ligand:peptide = 10:1); (d) compound 10 (ligand:peptide = 10:1).

5.3 Plate assay profiles and AFM images of different linkage types

We were interested in learning about the relationship between the molecular geometry of our scyllo-inositol derivatives and its effects on the morphology of Aβ aggregates formed. Our strategy employed oxime, amide, carbamate, and hydroxamate linkages while conserving the inositol and phenyl moieties.
Figure 5.4 Concentration dependent biotin-avidin Aβ1-42 oligomer assay profiles and AFM images of Aβ1-42. (a) oxime-linked; (b) amide-linked; (c) carbamate-linked. *P < 0.05 by ANOVA with Fisher PLSD.

The first three classes of linkages were evaluated by the oligomer assay and AFM as described by the above protocols (Figure 5.4). Qualitative analysis of the fluorescence data showed that the three compounds induced distinct concentration dependent profiles. The amide derivative (b) induced a decreased fluorescence at 50 nM and 50 μM. Conversely, the carbamate-linked derivative (c) showed significant increase in fluorescence at 5 nM, 50 nM, and 5 μM. The corresponding AFM images of Aβ1-42 peptide with each treatment produced less elongated Aβ1-42 fibrils compared to Aβ1-42 control (Figure 5.3, a). The subtle visual differences in Aβ morphology were not sufficient to conclude that these scyllo-inositol derivatives were inhibitory of Aβ1-42 fibrillogenesis. However, these compounds appear to actively modulate Aβ1-42 aggregation. Future work will reveal if these compounds prevent the formation of toxic Aβ oligomers with similar effectiveness to scyllo-inositol.
6 Conclusions

The purpose of this thesis was to develop a library of scyllo-inositol oximes as a strategy for further understanding the Aβ-scyllo-inositol binding interactions. We chose to functionalize the inositol backbone with a hydroxylamine in order to retain the key peripheral heavy atoms as our previous studies have demonstrated their importance for direct interactions with Aβ1-42.

The hydroxylamine starting material, 1-O-amino-scyllo-inositol hydrochloride, was successfully synthesized starting from myo-inositol via multistep protecting group transformations. This precursor readily conjugated with various aromatic aldehydes to rapidly generate a repertoire of oxime derivatives in good yields and high purity. This orthogonal reaction allows the divergent synthesis of countless derivatives for probing structure-activity relationships.

Hydroxamate-linked scyllo-inositol derivatives were also synthesized and the methodologies opened up a new class of related inositol compounds. These subtle differences in the covalent linkages, as well as the comparison with related oxime, would reveal whether the different linkages affect binding interactions. Although initial attempts to synthesize hydrazine derivatives were unsuccessful due to purification challenges, the reported efforts provide the groundwork for future optimizations. In addition to preparing mono-functionalized scyllo-inositol compounds, two inositol oligomers were synthesized. However, the poor solubility of these amphipathic inositol oligomers in common solvents limits their potential applications. Regardless, it is reasonable to speculate that Aβ1-42 oligomers have multiple binding sites for scyllo-inositol to form stabilized non-toxic species, therefore oligomeric inositols may potentially have improved avidity for Aβ1-42 oligomers.

Preliminary evaluation of several oxime derivatives using biotin-avidin Aβ1-42 oligomer assay and Aβ1-42 fibril analysis by AFM suggested that these compounds were active in the Aβ1-42 aggregation cascade. Phenyl oximes 8 and 10 promoted oligomerization more than the native scyllo-inositol suggesting the oxime linkage may be superior to hydroxamate, carbamate, or amide for the development of new inositol derivatives. Future in vitro experiments would indicate if these induced-oligomers are neurotoxic species or non-toxic as in the case of scyllo-
inositol. AFM analysis of Aβ_{1-42} fibrillogenesis also showed the two oximes modulated fibril growth. These initial results were encouraging as it opens up the possibility of discovering a new class of Aβ_{1-42} modulating compounds. A complete assessment of the oxime library and the other related inositol derivatives would provide a conclusive understanding of the molecular dynamics between scyllo-inositol and Aβ_{1-42} aggregates.
7 Experimental section

7.1 Generalities for organic synthesis

Reactions were monitored by thin layer chromatography using SiliaPlate Aluminium F$_{254}$ (Silicycle) with detection by quenching of fluorescence using a UV lamp (λ$_{max}$ = 254 nm) and/or by visualization with phosphomolybdic acid in ethanol (0.5% w/v). Silica column chromatography was performed on Silia-P Flash Silica Gel 60 (40-63 um particle size, Silicycle). RP-HPLC was performed on a Waters 1525 binary HPLC pump with a Waters 2487 dual λ absorbance detector, using VYDAC C-18 Monomeric (250 × 22 mm, Grace) column and eluted with CH$_3$CN/H$_2$O gradient at 10.0 mL/min. C-18 solid-phase extraction was performed using Bond Elut C-18 (6 CC/500 mg, Varian).

Reagents and solvents were purchased from Sigma Aldrich, Acros Organics, or AK Scientific and were used as supplied without further purification. Proton nuclear magnetic resonance spectra ($^1$H NMR) and carbon nuclear magnetic resonance spectra ($^{13}$C NMR) were recorded on a Varian Mercury 400 or Varian Mercury 300 NMR spectrometers. Chemical shifts for protons are reported in parts per million (δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvents (CHCl$_3$: δ 7.26, HDO: δ 4.79, CD$_2$HOD: δ 3.31, (CD$_3$)$_2$SO: δ 2.50). Chemical shifts for carbon resonances are reported in parts per million (δ scale) downfield from tetramethylsilane and are referenced to the carbon resonances of the solvents (CDCl$_3$: δ 77.2, CD$_3$OD: δ 49.0, (CD$_3$)$_2$SO: δ 39.5). Data are represented as follows: chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet), integration, coupling constant, and assignment. High resolution mass spectra were obtained with an ABI/Sciex Qstar mass spectrometer using electrospray ionization (ESI).
7.2 Preparation of hydroxylamino-scyllo-inositol

Compounds 1-4 were synthesized using previously established procedures in our laboratory. Spectral data for each compound conformed with literature.

\[
\begin{align*}
1,6:3,4\text{-Bis[O-(2,3-dimethoxybutane-2,3-diyl)]-2-ophthalimido-5-O-benzoyl-scyllo-inositol (5)}
\end{align*}
\]

A flame dried 25 mL round-bottom flask equipped with a stir bar was charged with triflate 4 (2.50 g, 3.88 mmol) and N-hydroxyphthalimide (785 mg, 4.81 mmol, 1.24 eq.), DMPU (7.5 mL) and triethylamine (0.67 mL, 4.81 mmol, 1.24 eq.). The red solution was stirred at 60 ºC for 16 h which became an orange solution. The crude mixture was transferred to a 150 mL round-bottom flask and water (75 mL) was added, resulting in the suspension of a white precipitate which was refrigerated at 4 ºC. The off-white solid was collected by vacuum filtration and washed with H₂O to remove residual DMPU. Silica gel chromatography (pentane/ethyl acetate = 2:1) afforded compound 5 as a white crystalline solid (1.94 mg, 76%); Rf 0.33 (pentane:ethyl acetate = 3:1); ¹H NMR (400 MHz, CDCl₃) δ 8.04 (d, 2H, J = 7.3), 7.81 (dd, 2H, J = 5.5, 3.0 Hz), 7.72 (dd, 2H, J = 5.5, 3.0 Hz), 7.55 (t, 1H, J = 7.3 Hz), 7.43 (dd, 2H, J = 7.3, 7.3 Hz), 5.43 (dd, 1H, J = 10, 10 Hz), 4.62 (dd, 1H, J = 10, 10 Hz), 4.06 (dd, 2H, J = 10, 10 Hz), 3.82 (dd, 2H, J = 10, 10 Hz), 3.37 (s, 6H), 3.11 (s, 6H), 1.18 (s, 12H); ¹³C NMR (75 MHz, CDCl₃) δ 165.1, 163.0, 134.4, 133.1, 130.2, 129.7, 129.3, 128.6, 123.3, 99.6, 99.5, 82.8, 69.6, 69.0, 68.7, 48.8, 47.8, 17.7, 17.4; HRMS m/z (ESI) calculated for C₃₃H₃₉NNaO₁₃ (M⁺Na)⁺ 680.2313; found: 680.2298.
1-\textit{O}-Phthalimido-4-\textit{O}-benzoyl-scyllo-inositol (6)

Compound 5 (300 mg, 0.456 mmol) was added to a solution of 95\% trifluoroacetic acid (5 mL) and stirred at 0 °C for 30 min. The yellow solution was warmed to room temperature and stirred an additional 2 h. Subsequently, the mixture was evaporated to dryness with N\(_2\) (g). Co-evaporation with ethanol (3 x 10 mL) under reduced pressure followed by evacuation under high vacuum overnight removed traces of trifluoroacetic acid and 2,3-butanedione to afford compound 6 as a white solid (194 mg, 98\%); R\(_f\) 0.37 (4\% MeOH in CH\(_2\)Cl\(_2\)); \(^1\)H NMR (400 MHz, (CD\(_3\))\(_2\)SO) \(\delta\) 7.97 (d, 2H, \(J = 7.5\) Hz), 7.89-7.84 (m, 4H), 7.64 (t, 1H, \(J = 7.5\) Hz), 7.51 (dd, 2H, \(J = 7.5, 7.5\) Hz), 5.23 (d, 2H, -OH, \(J = 5.5\) Hz), 5.13 (d, 2H, -OH, \(J = 4.8\) Hz), 4.88 (t, 1H, \(J = 9.8\) Hz), 4.16 (t, 1H, \(J = 9.2\) Hz), 3.59 (td, 2H, \(J = 9.6, 5.5\) Hz), 3.40 (td, 2H, \(J = 9.2, 4.8\) Hz); \(^{13}\)C NMR (100 MHz, (CD\(_3\))\(_2\)SO) \(\delta\) 165.2, 163.8, 134.6, 132.8, 130.5, 129.3, 128.7, 128.4, 123.2, 87.8, 75.5, 72.0, 71.3; HRMS \(m/z\) (ESI) calculated for C\(_{21}\)H\(_{20}\)NO\(_9\) (M\(^{+}\))\(^{1}\) 430.1132; found: 430.1137.

1-\textit{O}-Amino-scyllo-inositol hydrochloride (7)

A solution of compound 6 (194 mg, 0.452 mmol) and 2 M MeNH\(_2\) in anhydrous MeOH (5.5 mL, 11 mmol, 24 eq.) was stirred at room temperature for 48 h and a white precipitate was formed. The reaction mixture was evaporated to dryness with N\(_2\), followed by co-evaporation with MeOH under reduced pressure to remove residual MeNH\(_2\). The crude residue was acidified with aqueous concentrated HCl and dried to give a tan residue. The residue was added absolute ethanol (5 mL), swirled gently and decanted (repeated 4 times) to remove by-products N-methyl-phthalimide and methyl-benzamide. The white residue was added absolute ethanol (5 mL), sonicated, and cooled to -20 °C. Vacuum filtration afforded compound 7 as an off-white solid. The filtrate was concentrated, resuspended in absolute ethanol, and collected for a combined yield (62 mg, 59 \%); R\(_f\) 0.19 (EtOAc/MeOH/H\(_2\)O = 5:2:1); \(^1\)H NMR (400 MHz, D\(_2\)O) \(\delta\) 3.94 (app. t, 1H, \(J = 9.5\) Hz), 3.67 (app. t, 2H, \(J = 9.5\) Hz), 3.43 (app. t, 2H, \(J = 9.5\) Hz).
= 9.5 Hz), 3.35 (app. t, 1H, \( J = 9.5 \) Hz); \(^{13}\)C NMR (75 MHz, D\(_2\)O) \( \delta \) 85.4, 73.3, 73.1, 72.1; HRMS \( m/z \) (ESI) calculated for C\(_6\)H\(_{14}\)NO\(_6\) (M\(^+\))\(^+\) 196.0815; found: 196.0809.

### 7.3 Preparation of arylaldoximes

**N-(Phenyl) O-(scyllo-inosityl)-carbaldoxime (8)**

![Diagram of 8](image1)

To a solution of hydroxylamine 7 (5.0 mg, 0.022 mmol) in 1:1 THF:H\(_2\)O (0.6 mL) was added benzaldehyde (2.6 \( \mu \)L, 0.026 mmol, 1.2 eq.) and the mixture was stirred at room temperature for 1 h. The mixture was diluted with H\(_2\)O (15 mL) and extracted with Et\(_2\)O (3 x 15 mL). Evaporation under reduced pressure and lyophilisation afforded 8 as a white solid (5.6 mg, 90%); \( R_f \) 0.74 (EtOAc/MeOH/H\(_2\)O = 5:2:1); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 8.35 (s, 1H), 7.74-7.64 (m, 2H), 7.56-7.46 (m, 3H), 3.98 (app. t, 1H, \( J = 9.5 \) Hz), 3.81 (app. t, 2H, \( J = 9.5 \) Hz), 3.51-3.35 (m, 3H); \(^{13}\)C NMR (75 MHz, D\(_2\)O) \( \delta \) 151.4, 131.6, 130.8, 129.1, 127.4, 84.9, 73.8, 73.6, 71.3; HRMS \( m/z \) (ESI) calculated for C\(_{13}\)H\(_{18}\)NO\(_6\) (M\(^+\))\(^+\) 284.1128; found: 284.1119.

**N-(2,3-Dihydroxyphenyl) O-(scyllo-inosityl)-carbaldoxime (9)**

![Diagram of 9](image2)

To a solution of hydroxylamine 7 (10 mg, 0.043 mmol) in 2:1 MeOH:H\(_2\)O (1.2 mL) was added 2,3-dihydroxybenzaldehyde (7.6 mg, 0.055 mmol, 1.3 eq.) and stirred overnight at room temperature. The white mixture was concentrated under reduced pressure and re-dissolved in H\(_2\)O (5 mL). The product was purified by passing through a C-18 plug and eluted with H\(_2\)O (30 mL). Evaporation under reduced pressure and lyophilisation afforded 9 as an off-white solid (11 mg, 78%); \( R_f \) 0.62 (EtOAc/MeOH/H\(_2\)O = 5:2:0.5); \(^1\)H NMR (400 MHz, D\(_2\)O) \( \delta \) 8.42 (s, 1H), 6.90 (t, 1H, \( J = 8.0 \) Hz), 7.04-6.95 (m, 2H), 4.03 (app. t, 1H, \( J = 9.5 \) Hz), 3.73 (app. t, 2H, \( J = 9.5 \) Hz), 3.47-3.36 (m, 3H); \(^{13}\)C NMR (75 MHz, D\(_2\)O) \( \delta \) 152.2,
To a solution of hydroxylamine 7 (10 mg, 0.043 mmol) in 2:1 MeOH:H₂O (1.2 mL) was added 2,4-dihydroxybenzaldehyde (7.2 mg, 0.052 mmol, 1.2 eq.) and stirred overnight at room temperature. The light-pink mixture was concentrated under reduced pressure, re-dissolved in H₂O (20 mL), and extracted with EtOAc (4x 15 mL). The aqueous layer was concentrated and purified by passing through a C-18 plug and eluted with H₂O (30 mL). Evaporation under reduced pressure and lyophilisation afforded 10 as an off-white solid (11 mg, 78%); Rₐ 0.70 (EtOAc/MeOH/H₂O = 5:2:0.5); ¹H NMR (400 MHz, D₂O) δ 8.35 (s, 1H), 7.27 (d, 2H, J = 8.5 Hz), 6.52 (dd, 2H, J = 8.5, 2.0 Hz), 6.46 (d, 1H, J = 2.0 Hz), 3.98 (app. t, 1H, J = 9.5 Hz); ¹³C NMR (75 MHz, D₂O) δ 159.1, 157.5, 152.1, 132.5, 109.9, 108.5, 103.0, 85.1, 73.7, 73.5, 71.4; HRMS m/z (ESI) calculated for C₁₃H₁₈NO₈ (M+1)⁺ 316.1026; found: 316.1019.

N-(2,4-Dihydroxyphenyl) O-(scylo-inosityl)-carbaldoxime (10)

To a solution of hydroxylamine 7 (10 mg, 0.043 mmol) in 2:1 MeOH:H₂O (1.2 mL) was added 2,5-dihydroxybenzaldehyde (7.2 mg, 0.052 mmol, 1.2 eq.) and stirred overnight at room temperature. The light-yellow mixture was concentrated under reduced pressure, re-dissolved in H₂O (20 mL), and extracted with EtOAc (4x 15 mL). The aqueous layer was concentrated and purified by passing through a C-18 plug and eluted with H₂O (50 mL). Evaporation under reduced pressure and lyophilisation afforded 11 as an off-white solid (11 mg, 78%); Rₐ 0.67 (EtOAc/MeOH/H₂O = 5:2:0.5); ¹H NMR (400 MHz, (CD₃)₂SO) δ 9.26 (s, 1H), 8.89 (s, 1H), 8.29 (s, 1H), 6.92 (d, 1H, J = 2.5 Hz), 6.75-6.60 (m, 2H), 4.81 (m, 4H, -
OH), 4.74 (m, 1H, -OH), 3.67 (app t, 1H, J = 10 Hz), 3.41-3.35 (m, 2H), 3.09-2.97 (m, 3H); $^{13}$C NMR (75 MHz, D$_2$O) δ 149.8, 148.8, 146.0, 118.2, 118.1, 116.8, 112.7, 86.4, 74.3, 73.8, 71.4; HRMS m/z (ESI) calculated for C$_{13}$H$_{18}$NO$_8$ (M$^+$1)$^+$ 316.1026; found: 316.1020.

**N-(1-Naphthyl) O-(scyllo-inosityl)-carbaldoxime (12)**

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 1:1 THF:H$_2$O (0.6 mL) was added 1-napththaldehyde (5.7 μL, 0.042 mmol, 1.2 eq.) and stirred overnight at room temperature. The light-yellow solution was concentrated under reduced pressure and re-dissolved in 10% CH$_3$CN in H$_2$O (4 mL). The product was purified by RP-HPLC and lyophilisation afforded 12 as an off-white solid (11.7 mg, 70%); $^1$H NMR (300 MHz, D$_2$O) δ 8.99 (s, 1H), 8.56 (d, 1H, J = 8.0 Hz), 8.06 (t, 2H, J = 8.5 Hz), 7.85 (d, 1H, J = 7.0 Hz), 7.73-7.59 (m, 3H), 4.06 (app t, 1H, J = 9.5 Hz), 3.87 (app t, 2H, J = 9.5 Hz), 3.55-3.37 (m, 3H); $^{13}$C NMR (75 MHz, D$_2$O) δ 147.9, 133.5, 130.0, 129.9, 128.6, 128.3, 127.7, 127.1, 126.2, 125.4, 125.0, 86.4, 74.4, 73.9, 71.5; HRMS m/z (ESI) calculated for C$_{17}$H$_{20}$NO$_6$ (M$^+$1)$^+$ 334.1285; found: 334.1293.

**N-(4-Quinolyl) O-(scyllo-inosityl)-carbaldoxime (13)**

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 1:1 THF:H$_2$O (0.6 mL) was added 4-quinolinecarboxaldehyde (6.6 mg, 0.042 mmol, 1.2 eq.) and stirred overnight at room temperature. The crude mixture was concentrated under reduced pressure and re-dissolved in 10% CH$_3$CN in H$_2$O (7.5 mL). The product was purified by RP-HPLC and lyophilisation afforded 13 as an off-white solid (8.4 mg, 72%); $^1$H NMR (300 MHz, D$_2$O) δ 9.17 (s, 1H), 9.06 (d, 1H, J = 5.5 Hz), 8.69 (d, 1H, J = 8.5 Hz), 8.33 (d, 1H, J = 5.5 Hz), 8.25 (d, 1H, J = 8.5 Hz), 8.17 (app t, 1H, J = 7.5 Hz), 8.06-7.97 (m, 1H), 4.27 (app t, 1H, J = 9.5 Hz), 3.86 (app t, 2H, J = 9.5 Hz), 3.54-3.40 (m, 3H); $^{13}$C NMR (75 MHz, (CD$_3$)$_2$SO) δ
147.9, 145.2, 131.5, 128.5, 126.4, 125.6, 124.8, 119.8, 87.9, 74.3, 73.8, 71.5; HRMS m/z (ESI) calculated for C_{16}H_{19}N_{2}O_{6} (M^{+})^{+} 335.1237; found: 335.1255.

**N-(2-Furfuryl) O-(scyllo-inosityl)-carbaldoxime (14)**

To a solution of hydroxylamine 7 (7.8 mg, 0.034 mmol) in 1:1 THF:H_{2}O (0.6 mL) was added 2-furaldehyde (3.5 µL, 0.042 mmol, 1.2 eq.) and stirred overnight at room temperature. The light-yellow solution was concentrated under reduced pressure and re-dissolved in H_{2}O (2 mL). The product was purified by RP-HPLC and lyophilisation afforded 14 as an off-white solid in 2:1 E:Z mixture (3.6 mg, 39%); \(^1\)H NMR (300 MHz, D_{2}O) δ 8.21 (s, 1H, N=C-H), 7.67-7.65 (m, 2H, Ar, Ar'), 7.61 (s, 1H, N=C-H'), 7.37 (d, 1H, J = 3.5 Hz, Ar'), 6.85 (d, 1H, J = 3.5 Hz, Ar), 6.69-6.65 (m, 1H, Ar'), 6.64-6.58 (m, 1H, Ar), 4.06 (app t, 1H, J = 10 Hz, H-1'), 3.95 (app t, 1H, J = 10 Hz, H-1), 3.76 (app t, 2H, J = 10 Hz, H-2, H-6, H-2', H-6'), 3.52-3.33 (m, 3H, H-3, H-4, H-5, H-3', H-4', H-5'); \(^{13}\)C NMR (75 MHz, D_{2}O) δ 146.2, 145.5, 145.0, 141.5, 137.8, 119.3, 115.4, 112.6, 112.2, 86.1, 85.3, 73.7, 73.6, 71.7, 71.2; HRMS m/z (ESI) calculated for C_{11}H_{16}NO_{7} (M^{+}1)^{+} 274.0921; found: 274.0923.

**N-(4-Bromophenyl) O-(scyllo-inosityl)-carbaldoxime (15)**

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 2:1 MeOH:H_{2}O (0.9 mL) was added 4-bromobenzaldehyde (7.7 mg, 0.041 mmol, 1.2 eq.) and stirred overnight at room temperature. The white mixture was concentrated under reduced pressure and re-dissolved in 6:1 H_{2}O:CH_{3}CN (7 mL). The product was purified by RP-HPLC and lyophilisation afforded 15 as an off-white solid (9.0 mg, 71%); \(^1\)H NMR (300 MHz, D_{2}O) δ 8.30 (s, 1H), 7.67-7.57 (m, 4H), 3.98 (app t, 1H, J = 9.5 Hz), 3.80 (app t, 2H, J = 9.5 Hz), 3.50-3.35 (m, 3H); \(^{13}\)C NMR (75 MHz, (CD_{3})_{2}SO) δ 146.6, 131.9, 131.7, 128.5, 122.7, 86.5, 74.4, 73.8, 71.3; HRMS m/z (ESI) calculated for C_{13}H_{17}NO_{6}Br (M^{+}1)^{+} 362.0233; found: 362.0239.
**N-(3-Nitrophenyl) O-(scyllo-inosityl)-carbaldoxime (16)**

To a solution of hydroxylamine 7 (5.1 mg, 0.022 mmol) in 2:1 MeOH:H₂O (0.6 mL) was added 3-nitrobenzaldehyde (5.4 mg, 0.044 mmol, 1.3 eq.) and stirred overnight at room temperature. The white mixture was concentrated under reduced pressure and re-dissolved in 4:1 H₂O:CH₃CN (5 mL). The product was purified by RP-HPLC and lyophilisation afforded 16 as an off-white solid (5.2 mg, 72%); ¹H NMR (300 MHz, (CD₃)₂SO) δ 8.45 (s, 1H), 8.40 (s, 1H), 8.24 (dd, 1H, J = 8.0, 2.0 Hz), 8.06 (d, 1H, J = 8.0 Hz), 7.72 (app t, 1H, J = 8.0 Hz), 4.82 (d, 2H, J = 4.5 Hz, -OH), 4.75 (d, 1H, J = 3.0 Hz, -OH), 3.77 (app t, 1H, J = 10 Hz), 3.47-3.35 (m, 2H), 3.33-3.27 (m, 2H, -OH), 3.12-2.95 (m, 3H); ¹³C NMR (75 MHz, (CD₃)₂SO) δ 148.1, 145.8, 134.4, 132.7, 130.4, 123.9, 120.9, 87.0, 74.4, 73.9, 71.3; HRMS m/z (ESI) calculated for C₁₃H₁₇N₂O₈ (M⁺) 329.0979; found: 329.0980.

**N-(4-Hydroxyphenyl) O-(scyllo-inosityl)-carbaldoxime (17)**

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 2:1 MeOH:H₂O (0.9 mL) was added 4-hydroxybenzaldehyde (5.3 mg, 0.044 mmol, 1.3 eq.) and stirred overnight at room temperature. The white mixture was concentrated under reduced pressure and re-dissolved in 4:1 H₂O:CH₃CN (5 mL). The product was purified by RP-HPLC and lyophilisation afforded 17 as an off-white solid (6.7 mg, 64%); ¹H NMR (300 MHz, D₂O) δ 8.26 (s, 1H), 7.58 (d, 2H, J = 8.5 Hz), 6.95 (d, 2H, J = 8.5 Hz), 3.93 (app t, 1H, J = 9.0 Hz), 3.78 (app t, 2H, J = 9.0 Hz), 3.49-3.33 (m, 3H); ¹³C NMR (75 MHz, (CD₃)₂SO) δ 158.7, 147.4, 128.3, 123.4, 115.6, 85.8, 74.4, 73.9, 71.3; HRMS m/z (ESI) calculated for C₁₃H₁₈NO₇ (M⁺) 300.1077; found: 300.1089.
**N-(3,4-Dihydroxyphenyl) O-(scyllo-inosityl)-carbaldoxime (18)**

![Chemical Structure](image)

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 2:1 MeOH:H₂O (0.9 mL) was added 3,4-dihydroxybenzaldehyde (5.9 mg, 0.043 mmol, 1.2 eq.) and stirred overnight at room temperature. The light yellow solution was concentrated under reduced pressure and re-dissolved in H₂O (2.5 mL). The product was purified by RP-HPLC and lyophilisation afforded 18 as an off-white solid in 3:1 E:Z mixture (7.9 mg, 72%); ¹H NMR (400 MHz, D₂O) δ 8.20 (s, 1H, N=C-H), 7.68 (s, 1H, N=C-H'), 7.44 (d, 1H, J = 8.5 Hz, Ar') 7.34 (s, 1H, Ar'), 7.24 (d, 1H, J = 2.0 Hz, Ar), 7.11 (dd, 1H, J = 8.5, 2.0 Hz, Ar), 7.01-6.93 (m, 1H, Ar, Ar'), 4.01 (app t, 1H, J = 9.5 Hz, H-1'), 3.94 (app t, 1H, J = 9.5 Hz, H-1), 3.78 (app t, 2H, J = 9.5 Hz, H-2, H-6, H-2', H-6'), 3.51-3.35 (m, 3H, H-3, H-4, H-5, H-3', H-4', H-5'); ¹³C NMR (100 MHz, D₂O) δ 151.1, 147.6, 147.1, 146.9, 144.5, 143.7, 125.6, 124.3, 123.0, 121.5, 119.0, 116.4, 116.0, 114.0, 85.8, 84.7, 73.8, 73.6, 71.7, 71.3; HRMS m/z (ESI) calculated for C₁₃H₁₈NO₈ (M⁺1)^+ 316.1026; found: 316.1022.

**N-(2,3,4-Trihydroxyphenyl) O-(scyllo-inosityl)-carbaldoxime (19)**

![Chemical Structure](image)

To a solution of hydroxylamine 7 (8.0 mg, 0.035 mmol) in 2:1 MeOH:H₂O (0.9 mL) was added 2,3,4-trihydroxybenzaldehyde (6.7 mg, 0.043 mmol, 1.2 eq.) and stirred overnight at room temperature. The white mixture was concentrated under reduced pressure and re-dissolved in H₂O (2.5 mL). The product was purified by RP-HPLC and lyophilisation afforded 19 as an off-white solid (8.5 mg, 73%); ¹H NMR (300 MHz, D₂O) δ 8.35 (s, 1H), 6.89 (d, 2H, J = 8.5 Hz), 6.59 (d, 2H, J = 8.5 Hz), 4.00 (app t, 1H, J = 9.5 Hz), 3.74 (app t, 2H, J = 9.5 Hz), 3.52-3.33 (m, 3H); ¹³C NMR (75 MHz, (CD₃)₂SO) δ 148.8, 148.0, 146.0, 132.7, 119.6, 110.0, 107.6, 86.2, 74.3, 73.8, 71.4; HRMS m/z (ESI) calculated for C₁₃H₁₈NO₉ (M⁺1)^+ 332.0976; found: 332.0986.
7.4 Preparation of hydroxamates

1,6:3,4-Bis-[O-(2,3-dimethoxybutane-2,3-diyl)]-2-O-amino-5-O-benzoyl-scyllo-inositol (20)

A solution of phthalimide 5 (100 mg, 0.152 mmol) in 2 M MeNH₂ in anhydrous MeOH (4.0 mL, 8.0 mmol) was stirred at room temperature for 24 h and a white precipitate was formed. The reaction mixture was evaporated to dryness with N₂, followed by co-evaporation with MeOH under reduced pressure to remove residual MeNH₂. Silica gel chromatography (2% MeOH in DCM) afforded compound 20 as a white crystalline solid (68.7 mg, 86%); Rᵣ 0.5 (4% MeOH in DCM); ¹H NMR (300 MHz, CDCl₃) δ 8.04 (d, 2H, J = 7.5 Hz), 7.55 (app t, 1H, J = 7.5 Hz), 7.43 (app t, 2H, J = 7.5 Hz), 5.37 (app t, 1H, J = 9.5 Hz), 4.75 (br s, 2H), 3.90 (app. t, 2H, J = 9.5 Hz), 3.85-3.69 (m, 3H), 3.29 (s, 6H), 3.12 (s, 6H), 1.31 (s, 6H), 1.19 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 165.1, 133.0, 130.3, 129.7, 128.5, 99.6, 99.5, 81.4, 70.0, 68.9, 68.7, 48.2, 47.8, 17.8, 17.7; HRMS m/z (ESI) calculated for C₂₅H₃₇NO₁₁Na (M⁺Na)⁺ 550.2258; found: 550.2264.

1,6:3,4-Bis-[O-(2,3-dimethoxybutane-2,3-diyl)]-2-O-(N,N-dibenzoyl)amino-5-O-benzoyl-scyllo-inositol (21)

To an oven-dried 10 mL round-bottom flask containing a solution of hydroxylamine 20 (50 mg, 0.095 mmol) in anhydrous CH₂Cl₂ (1.0 mL) was added Et₃N (15 μL, 0.11 mmol, 1.2 eq.) and stirred at 0 °C for 20 min. A solution of benzoyl chloride (17 μL, 0.14 mmol, 1.5 eq.) in anhydrous CH₂Cl₂ (0.5 mL) was added to the stirring reaction dropwise at 0 °C and was allowed to stir to room temperature overnight. The crude mixture was evaporated under reduced pressure, followed by silica gel chromatography (1% MeOH in DCM).
Compound 21 was isolated as white solid (52 mg, 74%); R<sub>f</sub> 0.57 (pentane:ethyl acetate = 3:1); ¹H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.21-8.13 (m, 2H), 8.10-8.02 (m, 2H), 7.78 (dd, 2H, J = 8.0, 1.5 Hz), 7.70-7.61 (m, 1H), 7.60-7.33 (m, 8H), 5.48 (app t, 1H, J = 10.0 Hz), 4.27-4.21 (m, 3H), 3.90-3.79 (m, 2H), 3.16 (s, 6H), 3.13 (s, 6H), 1.29 (s, 6H), 1.19 (s, 6H); ¹³C NMR (75 MHz, CDCl<sub>3</sub>) δ 165.2, 161.9, 148.2, 134.1, 133.0, 130.6, 130.3, 129.9, 129.7, 128.8, 128.5, 128.4, 125.9, 99.6 (2), 79.9, 70.2, 69.2, 67.5, 48.0, 47.8, 17.7 (2); HRMS m/z (ESI) calculated for C<sub>39</sub>H<sub>45</sub>NO<sub>13</sub>Na (M<sup>+</sup>Na)<sup>+</sup> 758.2783; found: 758.2791.

1-O-benzamido-scyllo-inositol (23)

A suspension of compound 22 (50 mg, 0.068 mmol) in MeOH (1.5 mL) and CH<sub>2</sub>Cl<sub>2</sub> (0.3 mL) was added NaOMe/MeOH (0.5 mL) dropwise and stirred at room temperature for 24 h. The clear solution was diluted with MeOH (20 mL) and neutralized with Amberlite IR-120 H<sup>+</sup> exchange resins. The mixture was filtered by gravity filtration and rinsed with MeOH. Evaporation of MeOH under reduced pressure produced a white residue. ¹H NMR spectroscopy of the crude product showed an upfield shift of the H-5 peak, indicative of benzoyl removal. Subsequently, the crude product 22 was stirred with 95% TFA (2 mL) at 0 ºC for 3.5 h, followed by evaporation with N<sub>2</sub> flow and MeOH (3x 10 mL). The crude product was purified by silica gel chromatography (5% MeOH in DCM to 15% MeOH in DCM) to afford hydroxamate 23 as a white solid (10.4 mg, 52% over 2 steps); R<sub>f</sub> 0.66 (EtOAc/MeOH/H<sub>2</sub>O = 5:2:0.5); ¹H NMR (300 MHz, D<sub>2</sub>O) δ 7.76 (d, 2H, J = 7.0 Hz), 7.67 (app t, 1H, J = 7.0 Hz), 7.55 (app t, 2H, J = 7.0 Hz), 3.79 (app t, 1H, J = 9.5 Hz), 3.68 (app t, 2H, J = 9.5 Hz), 3.45 (app t, 2H, J = 9.5 Hz), 3.36 (app t, 1H, J = 9.2 Hz); ¹³C NMR (100 MHz, D<sub>2</sub>O) δ 170.4, 133.1, 130.4, 129.1, 127.5, 88.9, 73.4, 70.8; HRMS m/z (ESI) calculated for C<sub>13</sub>H<sub>18</sub>NO<sub>7</sub> (M<sup>+</sup>1)<sup>+</sup> 300.1077; found: 300.1074.
2,6-Bis[O-(scyllo-inosityl)oxime] 2,6-naphthalenedicarboxaldehyde (33)

A solution of hydroxylamine 7 (5.0 mg, 0.021 mmol, 2.4 eq.) and 2,6-naphthalene dicarboxaldehyde (1.6 mg, 8.7 μmol) in DMSO (0.5 mL) was stirred at room temperature for 24 h. The reaction mixture was quenched with H₂O (0.5 mL) and stirred further for 30 min after which a white precipitate formed. The precipitate was collected by vacuum filtration and washed with H₂O (10 mL) to afford di-oxime 33 as a white solid (yield not determined); ¹H NMR (400 MHz, (CD₃)₂SO) δ 8.36 (s, 2H), 8.06 (s, 2H), 7.97 (s, 1H), 7.95 (s, 1H), 7.86 (d, 1H, J = 1.0 Hz), 7.84 (d, 1H, J = 1.0 Hz), 4.85-4.79 (m, 8H, -OH), 4.77-4.75 (m, 2H, -OH), 3.75 (app t, 2H, J = 9.5 Hz), 3.50-3.41 (m, 4H), 3.12-2.99 (m, 6H); HRMS m/z (ESI) calculated for C₂₄H₃₁N₂O₁₂ (M⁺)⁺ 539.1871; found: 539.1881.

1,4-Bis[O-(scyllo-inosityl)oxime] 1,4-benzenedicarboxaldehyde (35)

A solution of hydroxylamine 7 (12 mg, 0.052 mmol, 2.0 eq.) and 1,4-benzene dicarboxaldehyde (3.5 mg, 0.026 mmol) in DMSO (1.0 mL) was stirred at room temperature for 24 h. The reaction mixture was quenched with H₂O (2.0 mL) and stirred further for 4 h after which a white precipitate formed. The precipitate was collected by vacuum filtration and washed with H₂O (10 mL) to afford di-oxime 35 as a white solid (yield not determined); ¹H NMR (400 MHz, (CD₃)₂SO) δ 8.22 (s, 2H), 7.66-7.64 (m, 4H), 4.82-4.77 (m, 8H, -OH), 4.75-4.73 (m, 2H, -OH), 3.72 (app t, 2H, J = 9.5 Hz), 3.46-3.37 (m, 4H), 3.10-2.96 (m, 6H); HRMS m/z (ESI) calculated for C₂₀H₂₉N₂O₁₂ (M⁺)⁺ 489.1715; found: 489.1711.
7.5 $^1$H NMR *scyllo*-inositol-Aβ$_{1-42}$ binding experiment sample preparation

7.5.1 Aβ$_{1-42}$ peptide

i. To an Eppendorf tube (1 mL) was added Aβ$_{1-42}$ (1.0 mg, 4500 MW) and dH$_2$O (100 μL)

ii. The white mixture was sonicated to give a fine, white suspension

iii. 0.6% aq. NH$_4$OH solution (50 μL) was added to dissolve the mixture

iv. Added dH$_2$O (50 μL) and 10 mM aq. NaN$_3$ (100 μL)

v. Added dH$_2$O (700 μL) to give 1.0 mL final solution (pH ~7-8)

vi. Sample was allowed to sit for 72 h at room temperature after which the peptide had transitioned from random coils to β-structure.

vii. The Aβ$_{1-42}$ sample was lyophilized overnight to give a white solid

7.5.2 *scyllo*-Inositol standard

*scyllo*-Inositol (2.8 mg) was dissolved in D$_2$O (5 mL) to give a 3.1 mM stock solution.

7.5.3 Tris hydrochloride standard

Tris hydrochloride (4.9 mg) was dissolved in D$_2$O (10 mL) to give a 3.1 mM stock solution.

7.5.4 NMR sample preparation

To a 5 mm standard 8” NMR tube containing treated Aβ$_{1-42}$ peptide (1.0 mg, 0.22 μmol) was added D$_2$O (560 μL) and sonicated to give a white mixture. Subsequently, this mixture was added 3.1 mM *scyllo*-inositol stock (70 μL) and 3.1 mM Tris-HCl stock (70 μL) to give a white mixture with final concentration of 0.31 mM for each component (peptide:*scyllo*-inositol:Tris-HCl 1:1:1).
Appendices
$^1$H NMR 400 MHz (CDCl$_3$, 25 °C)

$^{13}$C NMR 75 MHz (CDCl$_3$, 25 °C)
$^1$H NMR 400 MHz ((CD$_3$)$_2$SO, 25 °C)

$^{13}$C NMR 100 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 400 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^{1}H$ NMR 400 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^1$H NMR 400 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^{1}$H NMR 400 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^1$H NMR 400 MHz ((CD$_3$)$_2$SO, 25 °C)

$^{13}$C NMR 75 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 100 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz (D$_2$O, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 300 MHz ((CD$_3$)$_2$SO, 25 °C)

$^{13}$C NMR 75 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1\text{H NMR}$ 400 MHz (D$_2$O, 25 °C)

$^{13}\text{C NMR}$ 100 MHz (D$_2$O, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 75 MHz ((CD$_3$)$_2$SO, 25 °C)
$^1$H NMR 300 MHz (CDCl₃, 25 °C)

$^{13}$C NMR 75 MHz (CDCl₃, 25 °C)
$^1\text{H NMR}$ 300 MHz (CDCl$_3$, 25 °C)

$^{13}\text{C NMR}$ 75 MHz (CDCl$_3$, 25 °C)
$^1$H NMR 300 MHz (D$_2$O, 25 °C)

$^{13}$C NMR 100 MHz (D$_2$O, 25 °C)
$^1$H NMR 400 MHz ((CD$_3$)$_2$SO, 25 °C)

$^1$H NMR 400 MHz ((CD$_3$)$_2$SO, 25 °C)
References


60. Halkier, B. A.; Olsen, C. E.; Moller, B. L., The biosynthesis of cyanogenic glucosides in higher plants. The (E)- and (Z)-isomers of p-hydroxyphenylacetaldehyde oxime as


