Designed Glycopeptidomimetics Disrupt Protein–Protein Interactions Mediating Amyloid β-Peptide Aggregation and Restore Neuroblastoma Cell Viability

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ABSTRACT: How anti-Alzheimer’s drug candidates that reduce amyloid 1−42 peptide fibrillation interact with the most neurotoxic species is far from being understood. We report herein the capacity of sugar-based peptidomimetics to inhibit both Aβ1−42 early oligomerization and fibrillation. A wide range of bio- and physicochemical techniques, such as a new capillary electrophoresis method, nuclear magnetic resonance, and surface plasmon resonance, were used to identify how these new molecules can delay the aggregation of Aβ1−42. We demonstrate that these molecules interact with soluble oligomers in order to maintain the presence of nontoxic monomers and to prevent fibrillation. These compounds totally suppress the toxicity of Aβ1−42 toward SH-SYSY neuroblastoma cells, even at substoichiometric concentrations. Furthermore, demonstration that the best molecule combines hydrophobic moieties, hydrogen bond donors and acceptors, ammonium groups, and a hydrophilic β-sheet breaker element provides valuable insight for the future structure-based design of inhibitors of Aβ1−42 aggregation.

INTRODUCTION

Protein–protein interactions mediating protein aggregation concern at least 30 different proteins and are associated with more than 20 serious human diseases, including Alzheimer’s (AD), Parkinson’s disease, and type 2 diabetes mellitus. The accumulation of extra- or intracellular protein deposits, often referred to as amyloid, characterize these protein misfolding diseases. AD, which is the most common form of late-life dementia,1 is associated with accumulation of intraneuronal neurofibrillary tangles and extracellular “senile” plaques containing insoluble fibrils composed of 40- or 42-residue amyloid-β peptides (Aβ1−40 or Aβ1−42).2 Monomeric Aβ peptides convert into fibrils through a complex nucleation process involving the formation of various aggregated species such as soluble oligomers and protofibrils of increasing size.3−5 Structural studies have reported that oligomeric and fibrillar species share a β-sheet rich conformation;6−10 however, the structure of the different oligomeric species is far from being understood. Although Aβ1−42 is not the most abundant amyloid peptide produced in vivo, it is the major constituent of amyloid plaques and is far more aggregative and neurotoxic than Aβ1−40.11,12 Experimental evidence supports the hypothesis that low molecular weight oligomers are primarily responsible for the neurodegeneration observed in AD.2,11,13−16 However, the role of fibrils should not be neglected because they have been demonstrated not to be inert species but are able to generate damaging redox activity and promote the nucleation of toxic oligomers.17,18 Hence, it remains crucial to develop inhibitors that can reduce the prevalence of small transient oligomers and also prevent the formation of fibrils. Numerous compounds have been reported as inhibitors or modulators of Aβ1−42 aggregation. The main drawbacks of the described molecules that jeopardize their development as drug candidates are a lack of binding selectivity leading to a high risk for various side effects for dyes or polyphenol natural products.19 poor
bioavailability and high propensity to self-aggregate for peptide derivatives,\textsuperscript{20,21} and a general lack of information regarding their mechanism of action, and in particular on their effects on toxic oligomer formation.\textsuperscript{19–21} To our knowledge, rationally designed small and “druggable” pseudo- or nonpeptidic aggregation inhibitors have been very scarcely reported.\textsuperscript{22,23} Some of us have described retro-inverso peptide inhibitors of both early oligomerization and fibrillization.\textsuperscript{24}

We previously reported a novel class of glycopeptide derivatives based on two hydrophobic dipeptides (Ala-Val and Val-Leu) linked to a hydrophilic D-glucopyranosyl scaffold through aminoalkyl and carboxyethyl linkers in C1 and C6 positions, respectively (compound 1, Figure 1).\textsuperscript{24} These pentapeptide analogues were shown to modulate A\(_\beta\)-sheet breaker, playing a major role in preventing the interactions between A\(_\beta\) species and thus inhibiting the aggregation. The introduction of a carbohydrate in peptides can also have a multifaceted impact on the properties of these molecules, such as modulating the hydrophilicity/hydrophobicity balance and conferring resistance to proteolytic cleavage.\textsuperscript{25}

In order to further decrease the number of potential sites for proteolytic attack, we have now introduced peptidomimetics in the upper arm in the C6 position. A wide range of bio- and physicochemical techniques was then used in order to evaluate the activity of the synthesized small hydrosoluble peptidomimetic compounds on early oligomerization, fibrillization, and toxicity of A\(_\beta\)\(_{1-40}\) and also to identify the A\(_\beta\)\(_{1-42}\) species targeted by these molecules.

\section{RESULTS}

\textbf{Design.} As we have already demonstrated the superiority of the \(\beta\) configuration of the C1 anomeric carbon in our previously reported glycopeptides,\textsuperscript{24b} we decided in a first attempt to evaluate the mixture of \(\alpha\) and \(\beta\) anomers to avoid a difficult separation of the two anomers. Furthermore, as we have also clearly demonstrated the superiority of the amino propyloxy link relative to the amino ethyloxy link, in the C1 position of the sugar moiety,\textsuperscript{24b} we decided to prepare glycopeptidomimetics bearing the amino propyloxy link. For the design of the peptidomimetic strands, we chose to replace the C-terminal leucine (Leu5 in compound 1, Figure 1) by the 5-amino-2-methoxybenzhydrazide unit (compounds 2 and 3, Figure 1), which is a part of the \(\beta\)-strand mimic (“Hao” unit) reported by Nowick and co-workers.\textsuperscript{21,26} The introduction of a 5-amino-2-methoxybenzhydrazide unit into \(\beta\)-strand mimics was shown, by some of us, to be extremely effective in the prevention of protein-protein interactions involving intermolecular \(\beta\)-sheets of HIV-1 protease in order to inhibit its dimerization, while increasing the proteolytic stability of the molecules.\textsuperscript{27} In a first generation, the valine residue (Val4 in compound 1, Figure 1) was kept and linked to the 5-amino-2-methoxybenzhydrazide unit (compound 2, Figure 1). Next, the valine residue was replaced by a lysine residue, to further provide these molecules with the possibility of engaging in electrostatic interactions with A\(_\beta\)\(_{1-42}\) in order to increase their affinity for A\(_\beta\)\(_{1-42}\) (compound 3, Figure 1).

\textbf{Synthesis of the Glycopeptidomimetics.} A short and robust synthesis of intermediate 9 was developed (Scheme 1A). We started from the C1 allylic protected D-glucose which was transformed into 10 following the procedure described in the literature.\textsuperscript{28} The Michael addition of 4 on tert-butyl acrylate was performed to give 5. The allyl group of 5 was then removed from the C1 hydroxyl group with PdCl\(_2\) to give compound 6 in good yield. The anomeric hydroxyl of 6 was converted into the 5-amino-2-methoxybenzhydrazide unit (compound 7, Figure 1). Next, the valine residue was replaced by a lysine residue, to further provide these molecules with the possibility of engaging in electrostatic interactions with A\(_\beta\)\(_{1-42}\) in order to increase their affinity for A\(_\beta\)\(_{1-42}\) (compound 3, Figure 1).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Structure of glycopeptidomimetic derivatives 1–3.}
\end{figure}
The α and β epimers 8 were obtained in equal proportion and could not be separated at this stage. The tert-butyl group was finally cleaved in acidic conditions to give the carboxylic acid 9. Scaffold 9 was then coupled with the peptidomimetic arms 10 and 11 prepared according to our published procedure, using DMTMM ([4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate]) as coupling agent (Scheme 1B). Compounds 12 and 13 were obtained in good yield. The azido group of 12 and 13 was then reduced via a Staudinger reaction to give the corresponding amines, 14 and 15, in satisfactory yields. In order to build the peptidic arm in C1, the two amino acids N-Boc-L-Val-OH and N-Boc-L-Ala-OH were successively coupled by a standard coupling/deprotection protocol to afford 18 and 19 from 14 and 15, respectively, in

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good yields. Hydrogenolysis of 18 and 19 afforded 20 and 21, which underwent an acidic cleavage of the tert-butyl carbamate to give 2 and 3. The acidic cleavage of the tert-butyl carbamate was also performed on benzylated compounds 18 and 19 to afford 22 and 23. All of the desired compounds were obtained as a mixture of α and β anomers. The β anomer 3β was isolated after separation by HPLC.

**Inhibition of Aβ1−42 Fibrillation by Glycopeptidomimetics.** *ThT-Fluorescence Assays.* The ability of compounds 1−3 and of intermediates 19−23 to inhibit the fibrillation of Aβ1−42 was studied by ThT fluorescence spectroscopy. The fluorescence curve for Aβ1−42 at a concentration of 10 μM followed the typical sigmoidal pattern with a lag phase of 8−9 h followed by an elongation phase and a final plateau reached after 17−18 h (purple curve, Figure 2A). Two parameters were
derived from the ThT curves of $\text{A}\beta_{1-42}$ alone and $\text{A}\beta_{1-42}$ in the presence of the evaluated compound: (1) $t_{1/2}$, which is defined as the time at which the half maximal ThT fluorescence is observed and gives insight on the rate of the aggregation process; (2) $F$, the fluorescence intensity at the plateau, which is assumed to be dependent on the amount of fibrillar material formed (Table 1 and Figure 2A–C).

### Table 1. Effects of Compounds 1, 2, 3, $\beta$, 3β, 20, and 21 on $\text{A}\beta_{1-42}$ Fibrillation Assessed by ThT-Fluorescence Spectroscopy at a Compound/$\text{A}\beta_{1-42}$ ratio of 10/1 and 1/1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(compound/$\text{A}\beta$ ratio)</th>
<th>$t_{1/2}$ extension (%)</th>
<th>Plateau decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 10/1</td>
<td>280 ± 70</td>
<td>−56 ± 9</td>
<td></td>
</tr>
<tr>
<td>1 1/1</td>
<td>ne</td>
<td>ne</td>
<td></td>
</tr>
<tr>
<td>2 10/1</td>
<td>325 ± 12</td>
<td>−31 ± 7</td>
<td></td>
</tr>
<tr>
<td>2 1/1</td>
<td>155 ± 10</td>
<td>ne</td>
<td></td>
</tr>
<tr>
<td>3 10/1</td>
<td>NA</td>
<td>−87 ± 1</td>
<td></td>
</tr>
<tr>
<td>3 1/1</td>
<td>148 ± 12</td>
<td>−29 ± 9</td>
<td></td>
</tr>
<tr>
<td>3 0/1</td>
<td>ne</td>
<td>−23 ± 6</td>
<td></td>
</tr>
<tr>
<td>$\beta$ 10/1</td>
<td>NA</td>
<td>−90 ± 2</td>
<td></td>
</tr>
<tr>
<td>$\beta$ 1/1</td>
<td>165 ± 11</td>
<td>−34 ± 7</td>
<td></td>
</tr>
<tr>
<td>$\beta$ 0/1</td>
<td>129 ± 12</td>
<td>−16 ± 6</td>
<td></td>
</tr>
<tr>
<td>20 10/1</td>
<td>379 ± 15</td>
<td>−41 ± 22</td>
<td></td>
</tr>
<tr>
<td>20 1/1</td>
<td>138 ± 10</td>
<td>ne</td>
<td></td>
</tr>
<tr>
<td>21 10/1</td>
<td>NA</td>
<td>−84 ± 3</td>
<td></td>
</tr>
<tr>
<td>21 1/1</td>
<td>154 ± 8</td>
<td>−26 ± 6</td>
<td></td>
</tr>
</tbody>
</table>

*The concentration of $\text{A}\beta_{1-42}$ in this assay is 10 μM. The effect of 3 and $\beta$ at a compound/$\text{A}\beta$ ratio of 0.1/1 is also reported. ne = no effect; NA = no aggregation; parameters are expressed as the mean ± SE, $n = 3$–6. See Supporting Information for the calculation of the $t_{1/2}$ extension. A compound displaying a $t_{1/2}$ extension >100% delays aggregation. See Supporting Information for the calculation of the plateau decrease.

The glycopeptidomimetic molecules 2 and 3 were dramatically more efficient inhibitors of $\text{A}\beta_{1-42}$ aggregation than the glycopeptide compound 1 in particular at lower compound/$\text{A}\beta_{1-42}$ ratios of 1/1 and even 0.1/1. It is noteworthy that a lysine residue attached to the S-amino-2-methoxybenzhydrazide unit was highly beneficial for the activity compared to a valine residue (compare 3 vs 2 and 21 vs 20). The free amine of the lysine residue side chain is thus beneficial for the activity. However, no dramatic effect of the N-terminal free amine of the dipeptide Val-Ala chain was observed in both the lysine and valine series. Indeed, a similar activity was obtained for the free amine 3 and the Boc protected 21 on the one hand and for the free amine 2 and the Boc protected 20 on the other. It was also remarkable that the $\beta$ anomer $\beta$ showed a superior activity to the mixture of $\alpha$ and $\beta$ anomers in 3 at low compound/$\text{A}\beta_{1-42}$ ratios (1/1 and 0.1/1, Table 1 and Figures 2A–C). A supplementary ThT fluorescence assay was performed by first adding compound 3 after 4 h when presumably oligomers are already formed and second, adding compound 3 after 42 h when presumably essentially fibrils are present (Figure 1S). A similar activity was obtained with compound 3 added at the beginning of the kinetics or after 4 h. However, no effect (or even a slight increase of fluorescence) was observed when compound 3 was added after 42 h. As also observed in our previous glycopeptides series, benzylated derivatives 19 and 22–23 tended to self-aggregate and to slightly accelerate the aggregation process (Table 3S and Figure 1S), confirming that polar hydroxyl groups of the sugar moiety were essential to prevent the aggregation.

**TEM Experiments.** Transmission electron microscopy (TEM) analyses were performed on compound 3 that showed a more significant effect than 2 on $\text{A}\beta_{1-42}$ aggregation in the ThT-fluorescence assays. Images were recorded after 42 h of preincubation, corresponding to maximum aggregation in the ThT assays, with and without 3 (Figure 2D). Differences were observed regarding the amount of aggregates formed in the presence of 3 at both ratios. A very dense network of fibers displaying a typical morphology was observed for $\text{A}\beta_{1-42}$ alone. Only a few scattered, very short, and scarce fibers were visible on the grid containing the $\beta$ sample incubated with 3 at 10/1 ratio. This result validated the ThT-fluorescence data, indicating that compound 3 dramatically slowed down the aggregation of $\text{A}\beta_{1-42}$ (at 3/$\text{A}\beta_{1-42}$ ratio of 10/1) and efficiently reduced the amount of typical amyloid fibrils formed. It is noteworthy that even if at a 3/$\text{A}\beta_{1-42}$ ratio of 1/1 the fluorescence was not dramatically decreased in the ThT assays, but the morphology of the network observed by TEM was very different and less dense and that the sample contained some globular aggregates.

**Inhibition of $\text{A}\beta_{1-42}$ Oligomerization by Glycopeptidomimetics.** *Capillary Electrophoresis.* In order to determine their effect on small soluble oligomer formation, 3 and $\beta$ were studied by capillary electrophoresis (CE). We recently proposed an improved CE method to monitor easily over time the very early steps of the $\text{A}\beta_{1-42}$ oligomerization process. This technique has the advantage of being able to follow three kinds of soluble species, (i) the monomer (peak ES), (ii) different small metastable oligomers grouped under peak ES′, and (iii) transient species formed later and which correspond to species larger than dodecamers (peak LS). Aggregation kinetics of $\text{A}\beta_{1-42}$ alone showed that over time, the monomer ES peak decreased in favor of the oligomer peaks ES′ and LS, and of insoluble species, forming spikes in the profile (Figure 2S for the detailed kinetics). At time 0, the monomer peak ES was almost the only visible species, while after 12 h, only a small monomer peak remained, and many soluble aggregates, giving spikes, were present (Figure 3A).

In the presence of $\beta$ (3/$\text{A}\beta_{1-42}$ ratio of 1/1), the electrophoretic profile clearly indicated that the kinetics of aggregation was significantly slowed down. Indeed, $\beta$ maintained dramatically the presence of the monomer (peak ES). In addition, the large oligomer species grouped under the peak LS were still present at 12 h, while they completely disappeared in the control electrophoretic profile (Figure 3B and Figure 3S for the detailed kinetics). The preservation of the monomer was statistically significant; after 12 h, only 19% remained in the control experiment, while 52% remained in the presence of $\beta$ (Figure 3C). Similar results were observed with the mixture of $\alpha$ and $\beta$ anomers in 3; however, a slightly superior effect was observed for $\beta$ (41% of monomer species remained after 12 h in the presence of the mixture 3) (Figures 4S and 5S).

**Interaction of $\beta$ with Monomeric or Oligomeric Species of $\text{A}\beta_{1-42}$.** **NMR Experiments.** The goal of the NMR experiments was to study if compound $\beta$ was able to adopt any preferred conformation in solution and if it interacted in solution either with the monomeric species or with soluble aggregated forms of $\text{A}\beta_{1-42}$.

We first examined mixtures of $\text{A}\beta_{1-42}$ and $\beta$ at a temperature of 5 °C and using low concentrations of $\text{A}\beta_{1-42}$.
Figure 4. Interaction of 3β with Aβ1–42 monitored by NMR. Aromatic/amide (left) and aliphatic (right) regions of 1D 1H NMR spectra of 3β (0.4 mM) and Aβ1–42 (90 μM) at 5 °C. (A) Reference 1D 1H spectrum recorded at t = 0. (B–E) 1D 1H STD spectra recorded at t = 0 (B), after 2 days (C), 1 week (D), and 2.5 weeks (E). The assignment of the aromatic and methyl resonances of 3β is indicated. Amb means 5-amino-2-methoxybenzoyl. The signal marked with an asterisk corresponds to formic acid impurity.

The assignment of the aromatic and methyl resonances of 3β with an asterisk corresponds to formic acid impurity.

Figure 3. Effect of 3β on the early oligomerization steps by CE. Electrophoretic profile of Aβ1–42 peptide (100 μM) obtained immediately (0 h) and 12 h after sample reconstitution (10) alone (A) and in the presence of compound 3β at a compound/Aβ1–42 ratio of 1/1 (B). Results in the panel show the effect of 3β on the monomer ES (C). Results are the mean of 3 experiments.

(10–90 μM) to ensure that Aβ1–42 was mainly monomeric in freshly prepared samples.33 The 2D 1H−15N and 2D 1H−13C HSQC spectra of 10 μM 15N,13C-labeled Aβ1–42 recorded in the absence and in the presence of a large excess of 3β (0.4 mM) displayed no significant chemical shift perturbations of Aβ1−42 1H−15N and 1H−13C correlations (Figure 6S). Similarly, no chemical shift differences could be detected for the 1H signals of 3β in 1D 1H and 2D 1H−1H experiments (data not shown), even when higher concentrations of Aβ1−42 were used (up to 90 μM). Thus, NMR experiments demonstrated that 3β did not interact with monomeric Aβ1–42 peptide.

We then turned to magnetization transfer experiments that are commonly used to detect the binding of small ligands to large molecular weight species. Saturation transfer difference (STD) experiments were recorded to characterize binding properties and map binding epitopes of 3β.24,35 No STD signals could be detected in a control experiment with 3β alone, as expected for a low molecular weight molecule that did not aggregate in solution. The addition of Aβ1−42 peptide led to the appearance of weak STD signals (Figure 4). Interestingly, an increase in the STD signal was observed over time, reaching a maximum after 2.5 weeks. Concomitantly, a slow decay of the 1D 1H NMR signals of 3β was observed (Figure 7S), corresponding to the formation of high molecular weight Aβ1−42 aggregates that were too large to be observed by solution NMR spectroscopy.33 Thus, the gradual increase of the STD signal over several weeks could be explained by the slow conversion of monomeric Aβ1−42 to aggregated species that bind 3β. The STD signals were the strongest for the aromatic and methyl resonances of 3β, suggesting that the hydrophobic groups of the dipeptide and peptidomimetic strands were directly involved in the interaction with Aβ1−42 species.

WaterLOGSY experiments also enabled us to detect the binding of 3β to Aβ1–42 species through intermolecular magnetization transfers involving bulk water. The protons of 3β exhibited positive NOEs in the absence of Aβ1–42 (Figure 8S), as expected for a small molecule. The addition of Aβ1–42 caused a decrease of positive NOEs and a change of sign of the NOEs that became more negative over time, confirming that 3β binds to high molecular weight species in fast exchange on the NMR time scale.

Finally, NMR spectroscopy was used to analyze the structure of 3β in the free and bound forms. The 1D 1H NMR spectra of 3β alone were characterized by sharp line widths and concentration-independent chemical shifts (0.04–2 mM range), demonstrating that 3β was highly soluble and not prone to aggregation in the (sub) millimolar range. Chemical shifts, vicinal coupling constants, and ROEs analysis showed that the peptidic/pseudopeptidic arms and the aminooalkyl and carboxyethyl linkers were highly flexible, as supported by small
Intramolecular diasteroetric splitting of methylenic protons, averaged vicinal coupling constants (Table S1), intraresidual and sequential ROE intensities, and the absence of long-range ROEs. Furthermore, the amide protons exhibited strong temperature dependence of their chemical shifts (Table S1), which is an indicator of high solvent accessibility. Altogether, these NMR data indicated that 3β did not adopt per se hydrogen-bonded β-sheet conformations and had no self-association properties in solution. Interestingly, 2D NOESY experiments recorded on 3β in the presence of Aβ1-42 were characterized by modifications in the intensity of intraresidual and sequential NOEs which became more negative (Figure 9S). These changes correspond to transferred NOEs due to the transient binding of 3β to Aβ1-42 aggregated species. However, no additional long-range NOE correlations were detected, suggesting that 3β conformation remained largely extended and did not adopt a compact shape upon Aβ1-42 binding.

**SPR Experiments.** SPR was then used to evaluate the affinity between compound 3 and its β-anomer 3β and Aβ1-42 monomer bound to the gold surface.

To our knowledge, the few SPR experiments described in the literature to detect the affinity of ligands for Aβ1-42 have used either the depsipeptide molecule described by Taniguchi et al.36,37,22 or biotinylated Aβ1-42 immobilized onto streptavidin-coated chips.37 An SPR-based immunoassay has been also developed to recognize Aβ1-42 oligomers.39 The main drawbacks we found in these methods are the necessity to synthesize the noncommercial depsipeptide, the modest SPR response provided with these other approaches, and the use of modified peptides which may alter their affinity behavior. We thus developed a new method to immobilize the commercial Aβ1-42 peptide monomer by a classical peptide coupling through its amino groups. We paid particular attention to maintaining Aβ1-42 in its monomeric form upon immobilization. Recently, a similar method has been reported; however, no clear evidence on the nature of the immobilized species was provided.40

We optimized the immobilization of Aβ1-42 peptide by varying different parameters (pH and concentration of the sample preparation and injection parameters such as the flow, the time, and the number of injections). To ensure that only monomeric species were mainly immobilized, a rinsing step using an aqueous solution of NH₄OH·H₂O 0.1% was employed (see the procedure in Supporting Information), as we demonstrated previously by CE that these conditions were able to disaggregate oligomers and regenerate monomeric species.32 The characterization of the gold chip was performed using specific antibodies directed against the N- or C-term of Aβ1-42 (6E10 and MD 19-0016, respectively; see Supporting Information). Curcumin, which is a well-known disaggregant compound,41 did not lead to a decrease of the signal and was even found to bind to Aβ1-42 fixed on the SPR chips (Figure 18S). Finally, the affinity of ThT toward the peptide immobilized on the chip surface was evaluated before and after our optimized rinsing step, which used an aqueous solution of NH₄OH·H₂O 0.1%. Both SPR signal and fluorescence (visualized by fluorescence microscopy images of the channel) were higher before the rinsing step. The rinsing step is therefore crucial to disaggregate large species present initially on the chip surface in order to lead to a surface mainly composed by Aβ in its monomeric form (Figure 14S).

We conducted SPR measurements with compounds 3, 3β, and 1 to check their affinity for Aβ1-42 peptide. A concentration-dependent signal was observed; however, the response was very low in the range of the tested concentrations (up to 200 μM) indicating that these compounds have a very low affinity for the immobilized Aβ1-42 (Figures 15S, 16S, and 17S). This result is in accordance with the NMR data.

**Protection against Aβ1-42 Cell Toxicity.** The inhibitors were investigated to determine their ability to reduce the toxicity of aggregated Aβ1-42 to SH-SYSY neuroblastoma cells. The addition of either 1 or 3 showed a protective effect on cell survival (MTS assay, Figure 5) and membrane damage (LDH membrane integrity assay, Figure 19S) in the presence of cytotoxic 5 μM Aβ1-42. Remarkably, this protective effect was seen at equimolar amounts of inhibitor to Aβ1-42 and was still significant at a very low ratio of 0.1/1 (inhibitor/Aβ1-42) in the MTS assay.

**Plasma Stability.** The ability to withstand enzymatic cleavage in the circulatory system is an important requirement for any potential drug. Incubating the two inhibitors 1 and 3 in plasma gives an idea of how stable they will be once injected into the body. Compound 3 withstood 24 h at 37 °C with no obvious degradation in 10% plasma (Figure 20S). Compound 1 appeared to show some degradation over the same period, although the total area of the peaks did not change (Figure 20S). Unmodified polypeptides are usually degraded within minutes under these incubation conditions.

**DISCUSSION**

The introduction of a peptidomimetic strand based on a 5-amino-2-methoxybenzhydrazide unit linked through the carboxethyl in the C6 position of the d-glucopyranosyl scaffold not only increased the stability toward proteolytic degradation but also dramatically increased the capacity of these pentapeptide analogues to inhibit the fibrilization of Aβ1-42 as demonstrated by the ThT fluorescence and TEM experiments. The polar hydroxyl groups of the sugar moiety were essential to prevent the aggregation, as demonstrated by the lack of inhibitory activity of the benzyl analogues 19 and 22-23. A slightly superior effect was observed for the β anomer 3β compared to the mixture of α and β anomers in 3 (confirmed in the CE experiments). The presence of the amine of the side chain of the lysine residue in compound 3 proved to be beneficial for the inhibitory activity in comparison with the
Diverse Aβ monomer structures

Interaction of 3β (red) with Aβ oligomers

Hydrophobic Central or C-terminal part of Aβ

Ionic Interaction (Glu22/Asp23)

H Bonds and Hydrophobic Interaction

H Bonds and Hydrophobic Interaction

Polar and flexible β-breaker element:
Prevent association to additional Aβ peptide

Figure 6. Hypothesis of the mechanism of Aβ aggregation inhibition by 3β. (A) Proposed model of inhibition of the fibrilization of Aβ1−42 and the preservation of the Aβ1−42 monomer by 3β. (B) Proposed model of interaction of 3β with Aβ1−42.

valine residue in compound 2. This result suggests that an ionic interaction is likely to be established between this amine and acidic residues of Aβ1−42, strengthening the hydrophobic interactions involving aliphatic and aromatic moieties. Indeed, several computational and experimental studies on Aβ1−42 have shown that, in addition to the hydrophobic interactions involving in particular the 16−21 sequence (KLVFFA), the formation of a salt-bridge between amino acids Asp23 and Lys28 might stabilize a turn motif involving residues 24−28.42 An interaction with Glu22 might also be beneficial for the activity of the molecules.42b We can thus suggest, and this is supported by the NMR binding experiments (STD), that this novel class of glycopeptidomimetics is likely to interact through the hydrophobic sequences of the peptidomimetic and dipeptide sequences, presumably with a hydrophobic sequence of Aβ1−42 (such as the central K16−A21 or the C-terminal part I31−V40) and through an electrostatic interaction. The flexible and hydrophilic sugar moiety acts as a β-sheet breaker to prevent the aggregation. The effect of the glycopeptidomimetic on the early steps of oligomerization has been also demonstrated clearly by CE. Compound 3β dramatically preserved the nontoxic monomer of Aβ1−42 (ES). Oligomers larger than dodecamers (LS) were also stabilized. Both types of cell viability assays proved that preincubation of cytotoxic Aβ1−42 with glycopeptidomimetic 3 completely rescued the SH-SYSY neuroblastoma cells. The protective effect was observed even at substoichiometric concentrations (3 reduced cell death by 100% with 0.5 equiv and by 75% with 0.1 equiv in the MTS assay). This protective effect is much more pronounced than that observed with molecules which have undergone clinical trials, such as resveratrol,15 scylo-inositol,44 epigallocatechin-3-gallate (EGCG),44,45 or other molecules recently described as efficient reducers of Aβ1−42 toxicity.46 This effect is comparable to the best effect of the current Aβ aggregation inhibitors reported in the literature.22 Indeed, these molecules reduced Aβ1−42 toxicity only at stoichiometric or higher (5 to 10 equiv) concentrations. It is also noteworthy that glycopeptide 1 showed a dramatic effect on cell survival but was more sensitive to proteolytic attack.

The NMR and SPR experiments clearly indicated that this novel glycopeptidomimetic series does not bind to monomers with substantial affinity. NMR indicated that the Aβ1−42 species recognized by 3β are oligomeric forms whose concentration slowly increased with time. Thus, even if 3β is a small molecule that does not per se adopt a preferential conformation, it is able to recognize and bind to the early β-structured Aβ1−42 oligomers. The observation of magnetization transfers in STD, WaterLOGSY, and trNOESY experiments implied that the interconversion between the free and the Aβ1−42-bound forms of 3β occurred in fast exchange on the NMR time scale. We can thus hypothesize that such transient binding of 3β to oligomers may impede the subsequent addition of monomers or the association of oligomers into larger species and/or disrupt these early oligomers so that they revert back to monomers (Figure 6A).
Noteworthy, this inhibition effect is sequence-specific since compound 3 does not alter the kinetics of aggregation of another amyloid peptide, IAPP, involved in type 2 diabetes mellitus. Indeed, the peptidomimetic 3 does not inhibit the IAPP fibril formation even at a high peptidomimetic/IAPP ratio of 10/1 (see Supporting Information). The $t_{1/2}$ is not increased after the addition of 3, and the final fluorescence intensity remains the same.

## CONCLUSIONS

In conclusion, the present work validates the singular effect of sugar-based peptidomimetic analogues of pentapeptides on $\beta_{1-42}$ oligomerization and fibrillization. This new series has been designed in order to achieve three objectives: first, to engage hydrophobic, hydrogen bonds, and ionic interactions with $\beta_{1-42}$ thanks to small peptide and peptidomimetic arms; second, to prevent cross-$\beta$-sheet elongation of $\beta_{1-42}$ due to the hydrophilic sugar, considered as a $\beta$-sheet breaker element (Figure 6B). Finally, it has been designed also to be druggable, particularly to be a small molecule (MW around 800) with a particularly to be a small molecule (MW around 800) with a hydrophilic sugar, considered as a $\beta$-sheet breaker element.

Furthermore, this study reveals some remarkable properties of the $N$-42 oligomerization and $\beta$-sheet breakers, demonstrating again the potential of peptidomimetics as inhibitors of amyloid fibrillation.

## EXPERIMENTAL SECTION

### Chemistry. General Experimental Methods.

The usual solvents were purchased from commercial sources and dried and distilled by standard procedures. Compounds 4, 10, 11 and 17 were prepared according to published methods. Pure products were obtained after liquid chromatography using Merck silica gel 60 (40–63 $\mu$m). TLC analyses were performed on silica gel 60 F254 (0.26 mm thickness). The plates were visualized with UV light ($\lambda = 254$ nm) or revealed with a 4% solution of phosphomolybdic acid in EtOH. Mass spectra were obtained using a Bruker Esquire electrospray ionization apparatus. HRMS were obtained using a TOF LCT Premier apparatus (Waters), with an electrospray ionization source. The purity of compounds 2 and 19–23 was determined by HPLC using the 1260 Infinity system (Agilent Technologies) and a column SUNFIRE (C18, 5 $\mu$m, 150 mm $\times$ 2.1 mm); mobile phase for 3, acetoniure/H$_2$O + 0.2% formic acid from 1 to 100% in 20 min; mobile phase for 3B, acetoniure/H$_2$O + 0.2% formic acid at a ratio of 1/99 during 3 min, then gradient to 30/70 in 12 min; detection at 310 nm; flow rate, 0.25 ml/min.

### Spectroscopy.

$^1$H NMR (300 MHz, CD$_3$OD): $\delta$ = 9.50 (C1H, $\alpha$-proton, $\alpha$-methyl, $\alpha$-methylene, $\alpha$-amino, $\alpha$-ester, $\alpha$-amide), 77.9, 76.6, 75.0, 73.4, 72.5, 71.6, 71.1, 68.5, 68.4, 67.1, 60.7, 58.7, 50.1, 37.8, 37.6, 37.3 (C$_3$), 31.9, 30.0, 23.7, 19.7, 18.7, 17.7. HRMS (TOF, ESI, ion polarity positive, H$_2$O/MeOH): $m/z$ [M + H$^+$], calcd for C$_{36}$H$_{61}$N$_8$O$_{13}$ 813.4358; found 813.4352.

$^1$H NMR (300 MHz, CD$_3$OD): $\delta$ = 9.50 (C1H, $\alpha$-proton, $\alpha$-methyl, $\alpha$-methylene, $\alpha$-amino, $\alpha$-ester, $\alpha$-amide), 77.9, 76.6, 75.0, 73.4, 72.5, 71.6, 71.1, 68.5, 68.4, 67.1, 60.7, 58.7, 50.1, 37.8, 37.6, 37.3 (C$_3$), 31.9, 30.0, 23.7, 19.7, 18.7, 17.7. HRMS (TOF, ESI, ion polarity positive, H$_2$O/MeOH): $m/z$ [M + H$^+$], calcd for C$_{36}$H$_{61}$N$_8$O$_{13}$ 813.4358; found 813.4352.

$^1$H NMR (300 MHz, CD$_3$OD): $\delta$ = 9.50 (C1H, $\alpha$-proton, $\alpha$-methyl, $\alpha$-methylene, $\alpha$-amino, $\alpha$-ester, $\alpha$-amide), 77.9, 76.6, 75.0, 73.4, 72.5, 71.6, 71.1, 68.5, 68.4, 67.1, 60.7, 58.7, 50.1, 37.8, 37.6, 37.3 (C$_3$), 31.9, 30.0, 23.7, 19.7, 18.7, 17.7. HRMS (TOF, ESI, ion polarity positive, H$_2$O/MeOH): $m/z$ [M + H$^+$], calcd for C$_{36}$H$_{61}$N$_8$O$_{13}$ 813.4358; found 813.4352.
aqueous phase was extracted with EtOAc (3 x 60 mL). The combined organic layers were dried over Na2SO4, filtered, and concentrated under reduced pressure to give a crude oil which was purified by column chromatography on silica gel with eluent cyclohexane/EtOAc 80/20 to afford 5 as a colorless oil (7.1 g, 79%, α/β 70/30). Rf of cyclohexane/EtOAc 80/20). 1H NMR (300 MHz, CDCl3): δ = 7.34 (m, 1H); 5.93 (1H); 5.32 (dd, J = 17.2, 1.5 Hz, 1H); 5.22 (d, J = 10.0 Hz, 1H); 4.86 (d, J = 4.9 Hz, 0.75H, Hγ); 4.45 (d, J = 7.8 Hz, 0.25H, Hη); 5.10–4.54 (m, 6H, 4.25–3.47 (m, 10H); 2.52 (t, J = 6.3 Hz, 2H); 1.45 (s, 9H). 13C NMR (75 MHz, CDCl3): δ = 170.7, 130.9, 138.9, 138.2, 133.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5, 118.2, 95.7, 82.1, 80.5, 79.9, 77.6, 75.7, 75.0, 73.2, 70.2, 69.5, 76.2, 50.0, 42.1, 36.1, 31.0, 30.1, 27.8, 17.8, 17.4, 14.9. Anal. Caled for C31H31O4: C, 71.75; H, 7.65. MS [ESI, ion polarity positive, MeOH]: m/z: 641 [M + Na]+.

tert-Butyl 3-(3R,4S,5R,6R)-3,4,5-Tribenzyloxy-6-hydroxy-tetrahydropyran-2-yl)methoxypropanoate (6). To a stirred mixture of 5 (7.10 g, 11.47 mmol) in CH3OH/EtOH 2/1 (60 mL) was added HCl (10% in EtOH). The mixture was stirred under azote atmosphere overnight and became darker and finally black. The reaction mixture was filtered through a pad of Celite which was washed several times with CH2Cl2. The filtrate was then concentrated under reduced pressure to obtain a brown oil, which was purified by column chromatography on silica gel with eluent cyclohexane/EtOAc 90/10 to yield 6 as a yellow oil (4.61 g, 75%, α/β 70/30). Rf = 0.15 (cyclohexane/EtOAc 90/10). 1H NMR (300 MHz, CDCl3): δ = 7.32 (m, 15H); 5.32 (s, 0.70H, Hγ); 5.04–4.56 (m, 6.30H, 6H+HJ); 4.22–3.36 (m, 8H); 2.52 (t, J = 6.5 Hz, 2H); 1.45 (s, 9H). 13C NMR (75 MHz, CDCl3): δ = 170.7, 130.9, 138.9, 138.2, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5, 95.7, 82.1, 80.5, 79.9, 77.6, 75.7, 75.0, 73.2, 70.2, 69.5, 76.1, 36.1, 28.1. MS [ESI, ion polarity positive, MeOH]: m/z: 659 [M + Na]+.

tert-Butyl 3-(3R,4S,5R,6R)-3,4,5-Tribenzyloxy-6-(2,2,2-trichloro-ethyl)-imidoyl(oxy)-tetrahydropyran-2-yl)methoxypropanoate (7). To a solution of 6 (4.61 g, 7.96 mmol) in dry CH2Cl2 (80 mL) cooled at 0 °C was gradually added under azote atmosphere NaH (60% dispersion in mineral oil, 180 mg, 4.50 mmol). Then trichloroacetimidate (4.51 g, 7.96 mmol) in dry CH2Cl2 (80 mL) cooled at 0 °C was added as a white solid (593 mg, 86%, α/β 40/60); mp = 71–73 °C; Rf = 0.60 (CH3Cl/CH2Cl2 95/5). 1H NMR (300 MHz, CDCl3): δ = 12.05 (t, J = 6.2 Hz, 1H, NH); 11.28 (d, J = 0.6 Hz, 1H, NH); 9.80 (s, 1H); 8.58 (dd, J = 9.1, 2.4 Hz, 1H); 8.27 (d, J = 2.4 Hz, 1H); 7.33–7.19 (m, 15H); 7.07–6.95 (m, 2H); 5.30 (m, 1H); 4.94–4.58 (m, 4.60H, 6H+HJ); 4.40 (d, J = 7.8 Hz, 0.60H, Hγ); 4.02–4.34 (m, 15H); 2.59 (2H, 2.19 (s, 3H); 2.09–2.01 (m, 1H); 1.94–1.83 (m, 2H); 0.91–0.96 (m, 6H). 13C NMR (75 MHz, CDCl3): δ = 172.1, 171.1, 169.0, 164.9, 158.2, 158.1, 153.1, 139.8, 138.6, 138.4, 138.3, 138.2, 131.8, 133.9, 128.0, 128.0, 127.9, 127.8, 127.5, 124.7, 122.5, 117.7, 111.8, 103.6 (C1H); 97.1 (C2H); 84.6, 82.3, 81.9, 80.3, 77.8, 77.7, 75.5, 75.1, 75.0, 74.9, 73.1, 70.3, 70.1, 69.7, 67.7, 67.6, 66.6, 64.8, 55.4, 58.3, 43.7, 37.6, 37.5, 33.4, 33.3, 29.3, 28.9, 24.3, 18.9, 18.2. IR (neat): 3375 (N-H); 2979 (N-H) cm−1. Anal. Caled for C52H41O3N5O·0.5 H2O: C, 62.73; H, 6.58; N, 10.67; found: C, 62.78; H, 6.98; N, 10.85. MS [ESI, ion polarity negative, MeOH]: m/z 9080 [M+H]+.

Benzyl N-[5S]-6-(3-azidopropoxy)-3,4,5-tris-(benzoyloxy)-tetrahydropyran-2-yl)methoxypropanoic acid (8). To a solution of 7 as a white solid (593 mg, 68%, α/β 50/50). Rf = 0.15 (two diastereoisomers) (CH3Cl/CH2Cl2 95/5). 1H NMR (300 MHz, CDCl3): δ = 11.97 (sl, 1H, NH); 11.32 (sl, 1H, NH); 9.80 (sl, 1H, NH); 8.85 (d, J = 7.7 Hz, 1H, NH); 8.17 (m, 1H); 7.32–7.26 (m, 2H); 6.91 (m, 1H); 5.41 (m, 1H); 5.40–6.00 (m, 9.50H, 9H+HJ); 4.42 (d, J = 7.8 Hz, 0.50H, Hγ); 4.11–3.32 (m, 15H); 2.92 (m, 2H); 2.59 (2H, 2.21 (s, 3H); 188 (m, 1H, 1.37 (m, 6H). 13C NMR (75 MHz, CDCl3): δ = 174.2, 173.3, 169.2, 165.5, 165.3, 153.3, 133.8, 138.0, 137.8, 136.7, 133.8, 128.5, 128.0, 127.8, 125.0, 122.1 (Cβ), 117.7, 111.9, 103.6 (Cα), 97.2 (Cγ), 92.2, 87.2, 84.6, 82.0.
8.17, 75.6, 66.7 (C\text{18}), 56.4, 50.8, 48.3, 40.6, 37.6, 31.4, 30.4, 33.9, 29.1, 24.0, 22.2, 20.48. MS (ESI, ion polarity positive, MeOH): m/z: 1095.48 [M + Na]+.

**N-[((1S)-1-[(5-Acetamido-2-methoxy-benzoyl)amino]-carbamoyl]-2-methyl-propyl]-3-[(3R,4S,6R)-6-(3-amino-propoxy)-3,4,5-tribenzyloxy-tetrahydropyran-2-yl]methyl)-propanoic

**article**

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17.7. HRMS (TOF, ESI, ion polarity positive, H₂O/MeOH): m/z [M + Na]⁺, calcd for C₆₅H₈₅N₈O₁₅Na 913.4882; found 913.4883, m/z [M + Na]⁺, calcd for C₆₅H₈₅N₈O₁₅Na 935.4702, found 935.4708. HPLC purity: TR (α, β) = 12.8, 13.1 min, 89.9%.

(25)-N-[3-3RR,5S,6R]-6-[(25)-5-Acetamido-2-methoxy-benzoyl]-2-methyl-propyl]amino]-3-methyl-butanoyl]amino]propoxy]-6-oxo-hexyl]carbamate (23). The same procedure as that described for 16a from 18 (38 mg, 0.023 mmol) in dry CH₂Cl₂ (220 μL) to afford 23 as a white solid used (45 mg, quantitative, α/β 50/50), Rₛ = 0 (CH₂Cl₂/CH₂Cl₂: 95/5), 1H NMR (300 MHz, CDCl₃): δ = 11.0 (1H, s); 10.65 (1H, m); 9.52 (1H, m); 8.50–8.01 (1m, 7H); 7.32–7.25 (2m, 2H); 6.84 (1H, m); 5.25–4.49 (9m, 11H, 11H+H₂); 4.36 (m, 1.50H, 1H+H₂); 3.87 (s, 3H); 3.78–2.93 (16H), 2.51 (s, 2H); 2.11 (s, 3H); 1.96–1.81 (5m, 1H, 150 (s, 3H); 1.44 (m, 4H); 0.88–0.65 (8m, 6H). 13C NMR (75 MHz, CDCl₃): δ = 173.2, 172.0, 171.5, 171.3, 169.5, 165.8, 153.6, 138.6, 138.0, 136.6, 136.5, 135.9, 133.0, 128.4, 128.3, 127.9, 127.8, 127.7, 126.5, 128.9, 121.7, 116.9, 111.9, 103.9 (C₁₂H₁₅), 97.1 (C₁₂H₁₅), 84.4, 82.1, 81.8, 80.0, 78.2, 77.5, 74.7, 74.4, 74.2, 72.9, 69.6, 67.5, 66.5, 59.3, 59.1, 56.3, 53.0, 50.0, 49.6, 40.5, 36.7, 36.5, 36.2, 32.6, 31.4, 29.2, 23.9, 22.4, 19.0, 18.4, 17.3. HRMS (TOF, ESI, ion polarity positive, H₂O/MeOH): m/z [M + H]⁺, calcd for C₃₂H₃₀N₄O₨ Na 1239.5904; found 1239.5972. HPLC purity: TR (α, β) = 19.24, 19.67 min, 91.1%.

Fluorescence-Detected THT Binding Assay. ThT was obtained from Sigma. Aβ(1-42) was purchased from American Peptide. The peptide was dissolved in an aqueous 1% ammonia solution to a concentration of 1 mM and then, just prior to use, was diluted to 0.2 mM with 10 mM Tris-HCl and 100 mM NaCl buffer (pH 7.4). Stock solutions of glycopolymers were dissolved in DMSO with the final concentration kept constant at 0.5% (v/v).
TTT fluorescence was measured to evaluate the development of Aβ_{1-42} fibrils over time using a fluorescence plate reader (Fluostar Optima, BMG labtech) with standard 96-well black microtiter plates. Experiments were started by adding the peptide (final Aβ_{1-42} concentration equal to 10 μM) into a mixture containing 40 μM ThT in 10 mM Tris-HCl and 100 mM NaCl buffer (pH 7.4) with and without the tested compounds at different concentrations (100, 50, 10, 1 μM) at room temperature. The ThT fluorescence intensity of each sample (performed in duplicate or triplicate) was recorded with 440/485 nm excitation/emission filters set for 42 h performing a double orbital shaking of 10 s before the first cycle. The fluorescence assays were performed between 2 and 4 times on different days, with the same batch of peptide. The ability of compounds to inhibit/accelerate Aβ_{1-42} aggregation was assessed considering both the time of the half-life of aggregation (t_{1/2}) and the intensity of the experimental fluorescence plateau (F). The relative extension (or reduction) of t_{1/2} is defined as the experimental t_{1/2} in the presence of the tested compound relative to the one obtained without the compound and is evaluated as the following percentage: IFAβ = FAβ + compound/FAβ × 100. The decrease (or increase) of the experimental plateau is defined as the intensity of experimental fluorescence plateau observed with the tested compound relative to the value obtained without the compound and is calculated as the absolute value of the following percentage: IFAPβ = FAβ + compound/FAβ ×100 (a decrease is indicated with a (-) and an increase with a (+).

Transmission Electron Microscopy. Samples were prepared under the same conditions as those in the ThT-fluorescence assay. Aliquots of Aβ_{1-42} (1 μM) in 10 mM Tris-HCl and 100 mM NaCl NaCl, pH 7.4 (in the presence and absence of the tested compounds) were adsorbed onto 300-mesh carbon grids for 2 min, washed, and dried. The samples were negatively stained for 45 s on 2% uranyl acetate in water. After draining off the excess of staining solution and drying, images were obtained using a Zeiss 912 Omega electron microscope operating at an accelerating voltage of 80 kV.

Capillary Electrophoresis. Sample preparation: the commercial Aβ_{1-42} was dissolved upon reception in 0.16% NH₄OH (at 2 mg/mL) for 10 min at 20 °C, followed by an immediate lyophilization. The dried sample was then stored at −20 °C until use. CE experiments were carried out with a P/ACE TM MDQ Capillary Electrophoresis System (Beckman Coulter Inc., Brea, CA, USA) equipped with a photodiode array detector. UV detection was performed at 195 nm. The sample (as previously described) was reconstituted by dissolution in 20 mM phosphate buffer at pH 7.4 containing DMSO (control or stock solutions of glycopeptidomimetic dissolved in DMSO). A constant DMSO/phosphate buffer ratio at 2.5% (v/v) was used for each sample. The final peptide concentration was set at 100 μM regardless of the peptide/compound ratio. For the CE separation of Aβ oligomers, a fused silica capillary of 60 cm (10.2 cm to the detector) and 50 μm I.D. was used. The background electrolyte was a 80 mM phosphate buffer, pH 7.4. The separation was carried out under −20 kV at 25 °C. The sample was injected from the outlet by hydrodynamic injection at 0.5 psi for 10 s. After each run, the capillary was rinsed for 3.5 min with 1 M NaOH, 3.5 min with water, 1 min with DMSO 10%, 1 min with SDS 50 mM, and equilibrated with running buffer for 5 min.

NMR spectroscopy. NMR experiments were recorded on a Bruker Avance III 500 MHz spectrometer equipped with a 1H/13C/15N TCI cryoprobe with Z-axis gradient. NMR spectra were processed and analyzed with TopSpin software (Bruker).

The conformation of 3β was studied in aqueous solution, either in H₂O/D₂O (90/10 v/v) or in 50 mM sodium phosphate, pH 7.4 containing 10% D₂O. 1H and 13C resonances were assigned using 1D 1H Watergate, 2D 1H-1H TOCSY (MLEV17 isotropic scheme of 68 ms duration), 2D 1H-1H ROESY (500 ms mixing time), 2D 1H-13C HSQC, and 2D 1H-13C HMBC spectra. The 1H and 13C chemical shifts were calibrated using DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) as an internal reference. The 1H and 13C resonance assignments of 3β are listed in Tables S1 and S2. Vincinal coupling constants were extracted from 1D 1H Watergate. The temperature gradients of the amide proton chemical shifts were derived from 1D 1H Watergate spectra recorded between 5 and 30 °C.

Samples of Aβ_{1-42} in the absence or in the presence of 3β were prepared in Shigemi tubes (280 μL volume) in 50 mM sodium phosphate, pH 7.4, containing 10% D₂O. Synthetic Aβ_{1-42} peptide was used in NMR experiments, with the exception of 2D HSQC experiments requiring 15N and 13C-labeled recombinant Aβ_{1-42}. Solid phase peptide synthesis of Aβ_{1-42} was performed at the Institut de Biologie Intégrative (IFR83- Université Pierre et Marie Curie). Recombinant Aβ_{1-42} was obtained according to the protocol of Walsh et al. NMR experiments were acquired at 5 °C. 2D NOESY experiments were recorded with a mixing time of 0.2 s. 1H STD experiments were acquired using a cascade of Gaussian shape pulses (50 ms pulse, B1 field of 0.1 kHz, total duration of 3 s) applied on resonance (−0.7 ppm) and off resonance (±30 ppm), alternatively. The number of scans was set to 320, corresponding to an experiment duration of 50 min. 1H 1H WaterLOGSY (water-ligand observed via gradient spectroscopy) experiments were recorded using a Gaussian pulse of 20 ms duration for selective inversion of water magnetization and a mixing period of 0.5 s. The recycling delay was set to 2 s, and the total number of scans was 1200, corresponding to an experimental time of 1 h.

SPR Experiments. For these studies, we used the Biacore T100 (GE Healthcare, France) apparatus, which has 4 parallel flow channels. Aβ_{1-42} peptide was immobilized on the carboxy-terminated dextran matrix on a gold surface sensor chip (CMS sensor chip, GE Healthcare, France) by an optimized amine coupling method. Briefly, the surface was treated with a mixture of 0.4 M EDC and 0.1 M NHS (1/1) in water for 7 min at 10 μL/min. Then, a freshly prepared Aβ_{1-42} solution (0.05 μM) in 10 mM sodium acetate buffer (pH 4.6) was injected 4 times during 15 min each at 10 μL/min on the NHS-activated surface. Then, a final injection of ethanolamine was done to block the nonlinked activated amine. A reference surface was prepared using the same immobilization procedure but with an ethanolamine injection instead of the peptide (blank surface). At that point of the process, the fixation led to a SPR signal of 3000 RU. A rinsing step using an aqueous solution of NH₄OH-H₂O 0.1% (9 injections of 1 min each at 30 μL/min) was performed in order to remove from the surface all of the Aβ_{1-42} aggregates that may have been formed during the immobilization step. After these rinsing steps, the chip gave a signal of about 1500 RU.

Binding of compounds 3β, I, and curcumin to Aβ_{1-42} fixed on the SPR chips: Solutions of compounds in 150 mM PBS containing 2% DMSO solubilized in DMSO were injected on the Aβ_{1-42} surface for 1 min at 80 μL/min. The rinsing step was performed using the running buffer for 5 min and the regeneration step using the aqueous solution of NH₄OH-H₂O 0.1% (3 times for 5 min at 30 μL/min). The range of concentrations started from 12.5 μM and ended at 200 μM in PBS (150 mM) and 2% DMSO. A DMSO solvent correction was applied to the raw signals, and the nonspecific signal was subtracted using the blank channel.

Cell Toxicity. SH-SY5Y neuroblastoma cells were grown in low serum OptiMEM (Life Technologies) for 24 h at 37 °C, 5% CO₂ in a 96 well plate at 20 000 cells per well. Aβ_{1-42} was dissolved in sterile PBS at 50 μM concentration in the presence of 1, 5, 10, and 50 μM of either 1 or 3 for 24 h at room temperature, along with a control incubation with no inhibitor. After the 24 h period, media were removed from the cells and replaced with OptiMem containing the preincubated Aβ_{1-42} plus inhibitor diluted one in ten (5 μM Aβ final concentration) in quadruplicate. The cells were incubated for a further 24 h as before, and the cell viability (MTS assay) and cell proliferation (LDH assay) assessed using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) and CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega), respectively. The assays were repeated twice, and representative samples are shown.

Plasma Stability. Both 1 and 3 were dissolved at 40 μM in DMSO and then diluted to 1 μM in 10% human plasma, 90% sterile PBS. One hundred microliter samples were run on an HPLC system (Dionex, with C18 Jupiter column from Phenomenex) using a gradient of 0–80% B (buffer A, 0.1% trifluoroacetic acid in water; buffer B, 0.05%
trifluoroacetic acid in acetonitrile). Samples were monitored at 230 nm. After 24 h of incubation at 37 °C, 100 μL samples were run on the same gradient and monitored as before.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01629.

NMR assignments of 3β; representative NMR spectra and HPLC purities, experimental procedure for fluorescence-detected ThT binding assay on Aβ40-42 and IAPP; representative curves of ThT fluorescence assays; experimental procedure for TEM, CE, NMR, and SPR (PDF)

SMILES data (CSV)

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**ABBREVIATIONS USED**

Aβ, amyloid-beta peptide; AD, Alzheimer’s disease; CE, capillary electrophoresis; STD, saturation transfer difference; ThT, thioflavin T; TEM, transmission electron microscopy; SPR, surface plasmon resonance; SAR, structure-activity relationship; DMTMM, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate]; DMAP, 4-dimethylaminopyridine

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