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Designed glycopeptidomimetics disrupt protein-protein interactions mediating amyloid β -peptide aggregation and restore neuroblastoma cell viability

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KEYWORDS. amyloid β -peptide, Alzheimer's disease, peptidomimetics, glycopeptides, aggregation, oligomers, capillary electrophoresis, nuclear magnetic resonance, surface plasmon resonance

ABSTRACT. How anti-Alzheimer's drug candidates that reduce amyloid 1-42 peptide fibrillization interact with the most neurotoxic species is far from being understood. We report herein the capacity of sugar-based peptidomimetics to inhibit both $A\beta_{1-42}$ early oligomerization and fibrillization. A wide range of bio- and physico-chemical 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 $A\beta_{1-42}$. We demonstrate that these molecules interact with soluble oligomers in order to maintain the presence of non-toxic monomers and to prevent fibrillization. These compounds totally suppress the toxicity of $A\beta_{1-42}$ towards SH-SY5Y neuroblastoma cells, even at sub-stoichiometric 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 $A\beta_{1-42}$ 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

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3 protein deposits, often referred to as amyloid, characterize these protein misfolding diseases. AD,
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5 which is the most common form of late-life dementia,¹ is associated with accumulation of
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7 intraneuronal neurofibrillary tangles and extracellular 'senile' plaques containing insoluble
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9 fibrils composed of 40 or 42-residue amyloid- β peptides ($A\beta_{1-40}$ or $A\beta_{1-42}$).² Monomeric $A\beta$
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11 peptides convert into fibrils through a complex nucleation process involving the formation of
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13 various aggregated species such as soluble oligomers and protofibrils of increasing size.³⁻⁵
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15 Structural studies have reported that oligomeric and fibrillar species share a β -sheet rich
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17 conformation,⁶⁻¹⁰ however the structure of the different oligomeric species is far from being
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19 understood. Although $A\beta_{1-42}$ is not the most abundant amyloid peptide produced *in vivo*, it is the
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21 major constituent of amyloid plaques and is far more aggregative and neurotoxic than $A\beta_{1-40}$.^{11,12}
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23 Experimental evidence supports the hypothesis that low molecular weight oligomers are
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25 primarily responsible for the neurodegeneration observed in AD.^{2,11,13-16} However, the role of
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27 fibrils should not be neglected, because they have been demonstrated not to be inert species, but
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29 are able to generate damaging redox activity and promote the nucleation of toxic oligomers.^{17,18}
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31 Hence it remains crucial to develop inhibitors that can reduce the prevalence of small transient
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33 oligomers and also prevent the formation of fibrils. Numerous compounds have been reported as
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35 inhibitors or modulators of $A\beta_{1-42}$ aggregation. The main drawbacks of the described molecules
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37 that jeopardize their development as drug candidates are: a lack of binding selectivity leading to
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39 a high risk for various side-effects for dyes or polyphenol natural products¹⁹; poor bioavailability
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41 and high propensity to self-aggregate for peptide derivatives^{20,21}; and a general lack of
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43 information regarding their mechanism of action, and in particular on their effects on toxic
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45 oligomers formation.¹⁹⁻²¹ To our knowledge, rationally designed small and 'druggable' pseudo-
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3 or non-peptidic aggregation inhibitors have been very scarcely reported.^{22,23} Some of us have
4 described retro-inverso peptide inhibitors of both early oligomerization and fibrillization.²²
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8 We previously reported a novel class of glycopeptide derivatives, based on two hydrophobic
9 dipeptides (Ala-Val and Val-Leu) linked to a hydrophilic D-glucopyranosyl scaffold through
10 aminoalkyl and carboxyethyl linkers in C1 and C6 positions, respectively (compound **1**, Figure
11 1).²⁴ These pentapeptide analogs were shown to modulate A β ₁₋₄₀ and A β ₁₋₄₂ aggregation, as
12 demonstrated by fluorescence Thioflavin-T (ThT) assays and transmission electron microscopy
13 (TEM).²⁴ The flexible and hydrophilic sugar moiety is believed to act as a β -sheet breaker,
14 playing a major role in preventing the interactions between A β species and thus inhibiting the
15 aggregation. The introduction of a carbohydrate in peptides can also have a multifaceted impact
16 on the properties of these molecules, such as modulating the hydrophilicity/hydrophobicity
17 balance and conferring resistance to proteolytic cleavage.²⁵
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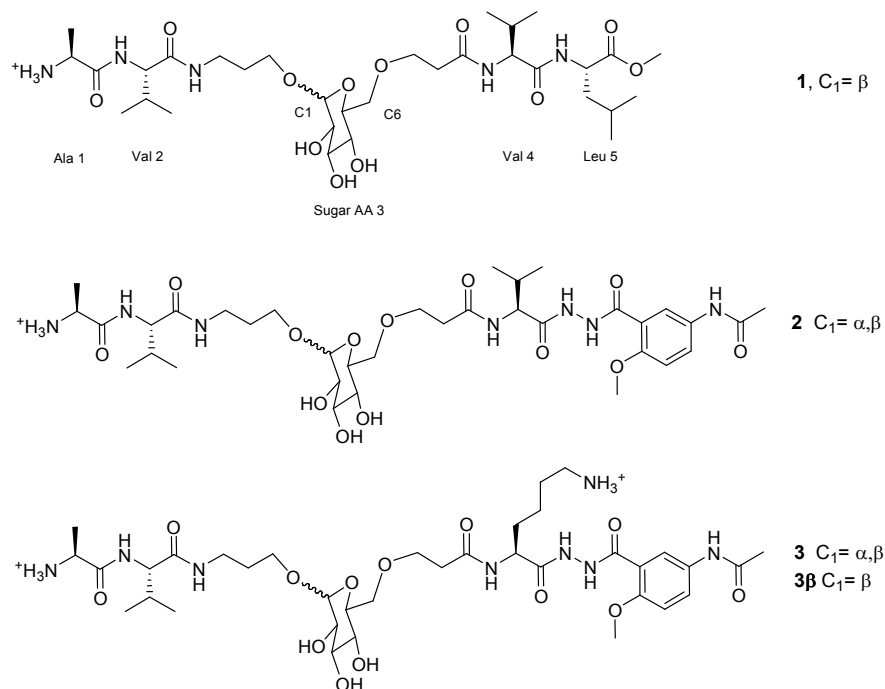
32 In order to further decrease the number of potential sites for proteolytic attack, we have now
33 introduced peptidomimetics in the upper arm in the C6 position. A wide range of bio- and
34 physico-chemical techniques was then used in order to evaluate the activity of the synthesized
35 small hydrosoluble peptidomimetic compounds on the early oligomerization, fibrillization and
36 toxicity of A β ₁₋₄₂ and also to identify the A β ₁₋₄₂ species targeted by these molecules.
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45 **RESULTS**

46 **Design**

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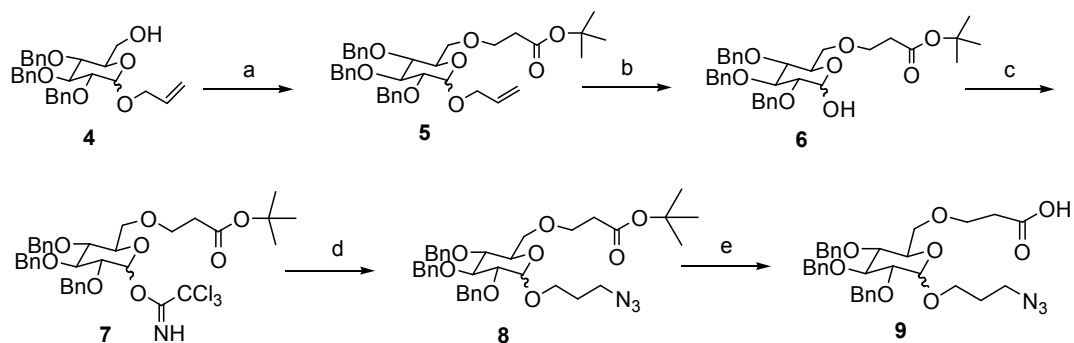
28 **Figure 1.** Structure of glycopeptidomimetic derivatives 1-3

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31 **Synthesis of the glycopeptidomimetics**

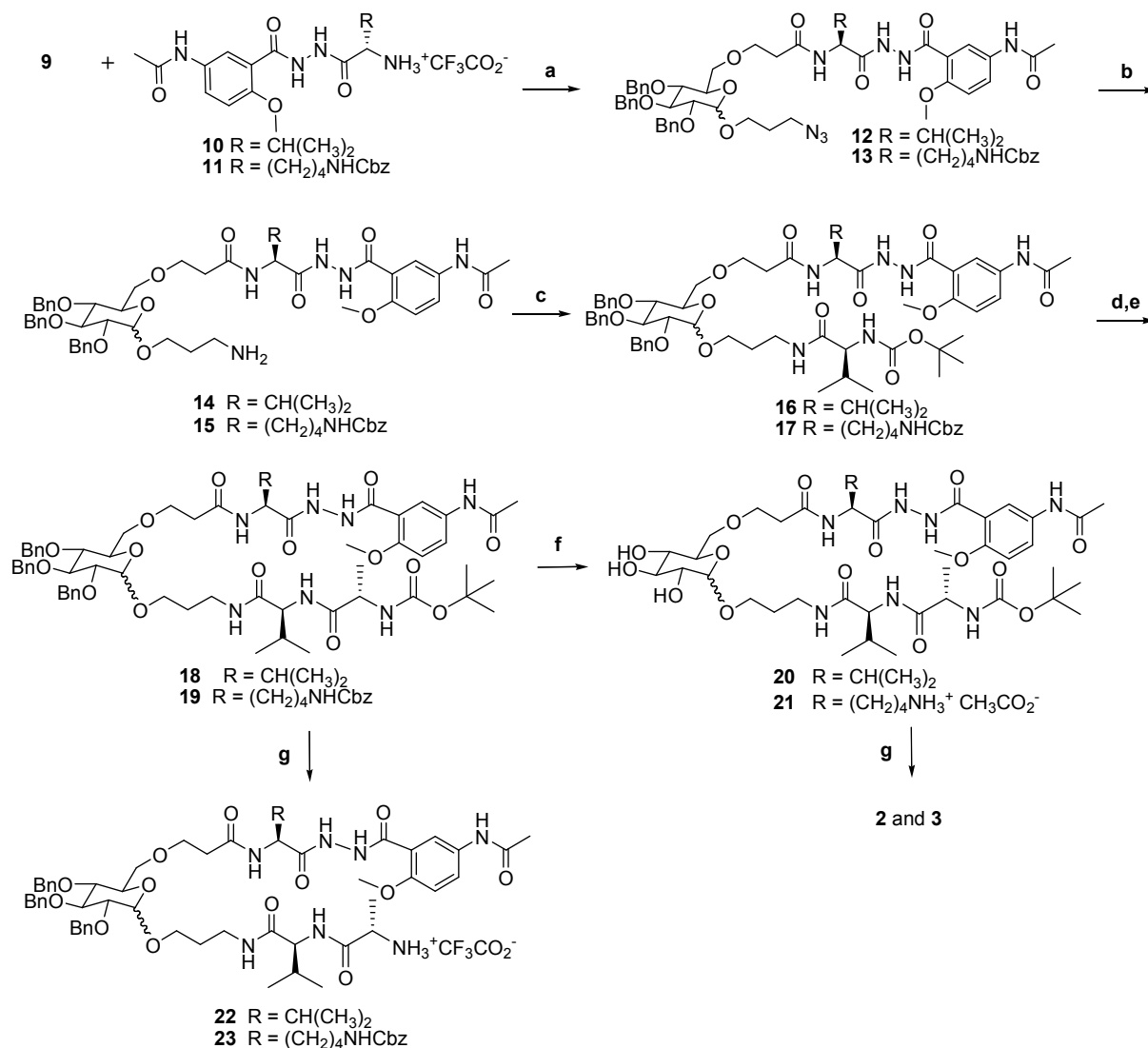
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34 A short and robust synthesis of the intermediate **9** was developed (Scheme 1A). We started
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36 from the C1 allylic protected D-glucose which was transformed into **4** following the procedure
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38 described in the literature.²⁸ The Michael addition of **4** on tert-butylacrylate was performed to
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40 give **5**. The allyl group of **5** was then removed from the C1 hydroxyl group with PdCl₂ to give
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42 compound **6** in good yield. The anomeric hydroxyl of **6** was converted into the
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44 trichloroacetimidate intermediate **7**, in the presence of trichloroacetonitrile and using NaH as a
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46 base. The nucleophilic substitution reaction by 3-azidopropan-1-ol was then carried out in the
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48 presence of AuCl (10%) affording **8** in good yield. The α and β epimers **8** were obtained in equal
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50 proportion and could not be separated at this stage. The tert-butyl group was finally cleaved in
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52 acidic conditions to give the carboxylic acid **9**.
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3 The scaffold **9** was then coupled with the peptidomimetic arms **10** and **11** prepared according to
4 our published procedure²⁷, using DMTMM ([4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-
5 morpholinium tetrafluoroborate])²⁹ as coupling agent (Scheme 1B). Compounds **12** and **13** were
6 obtained in good yield. The azido group of **12** and **13** was then reduced via a Staudinger
7 reaction³⁰ to give the corresponding amines, **14** and **15** in satisfactory yields. In order to build the
8 peptidic arm in C1, the two amino acids *N*-Boc-L-Val-OH and *N*-Boc-L-Ala-OH were
9 successively coupled by a standard coupling/deprotection protocol to afford **18** and **19** from **14**
10 and **15** respectively, in good yields. Hydrogenolysis of **18** and **19** afforded **20** and **21**, which
11 underwent an acidic cleavage of the *tert*-butyl carbamate to give **2** and **3**. The acidic cleavage of
12 the *tert*-butyl carbamate was also performed on benzylated compounds **18** and **19** to afford **22**
13 and **23**. All the desired compounds were obtained as a mixture of α and β anomers. The β anomer
14 **3 β** was isolated after separation by HPLC.
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A



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Scheme 1. Synthesis of glycopeptidomimetics. A- Synthesis of the scaffold **9**. Reagents and conditions: a) *tert*-butyl acrylate, TBAB, 20% NaOH aq., rt, 24h, 79%; b) PdCl₂, CH₃OH/EtOH, N₂ atm. rt, overnight 75% ; c) CCl₃CN, NaH, CH₂Cl₂, rt, overnight, 75%; d) 3-azidopropan-1-ol, AuCl (10% w/w), CH₂Cl₂, N₂ atm., rt, 2 days, 82%; e) TFA, CH₂Cl₂, rt, overnight, 72%. B- Synthesis of glycopeptidomimetics **2** and **3**. Reagents and conditions: a) NMM, DMTMM, DMF, rt, overnight, 86% (**12**), 68%, (**13**); b) Ph₃P, THF/H₂O (9:1), 40 °C, 24h, 63% (**14**), 50% (**15**); c) *N*-Boc-L-Val-OH, NMM, DMTMM, DMF, rt, overnight, 79% (**16**), 68% (**17**); d) TFA, CH₂Cl₂,

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3 rt, 3h, quantitative; e) *N*-Boc-L-Ala-OH, NMM, DMTMM, rt, overnight, 68% (**18**), 73% (**19**); f)
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5 H₂ Pd/C, rt, MeOH, 48h, 88% (**20**); 75% (**21**); g) TFA, CH₂Cl₂, rt, 3h, quantitative.
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8 9 **Inhibition of A β ₁₋₄₂ fibrillization by glycopeptidomimetics**

10 11 *ThT-fluorescence assays*

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13 The ability of compounds **1-3** and of intermediates **19-23** to inhibit the fibrillization of A β ₁₋₄₂
14 was studied by ThT fluorescence spectroscopy.³¹ The fluorescence curve for A β ₁₋₄₂ at a
15 concentration of 10 μ M followed the typical sigmoidal pattern with a lag phase of 8–9 h
16 followed by an elongation phase and a final plateau reached after 17-18 h (purple curve, Figure
17 2A). Two parameters were derived from the ThT curves of A β ₁₋₄₂ alone and A β ₁₋₄₂ in the
18 presence of the evaluated compound: (1) $t_{1/2}$, which is defined as the time at which the half
19 maximal ThT fluorescence is observed and gives insight on the rate of the aggregation process;
20 (2) F, the fluorescence intensity at the plateau which is assumed to be dependent on the amount
21 of fibrillar material formed (Table 1, Figures 2A-C).
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35 The glycopeptidomimetic molecules **2** and **3** were dramatically more efficient inhibitors of
36 A β ₁₋₄₂ aggregation than the glycopeptide compound **1** in particular at lower compound/ A β ₁₋₄₂
37 ratios of 1/1 and even 0.1/1. It is noteworthy that a lysine residue attached to the 5-amino-2-
38 methoxybenzhydrazide unit was highly beneficial for the activity compared to a valine residue
39 (compare **3** vs **2** and **21** vs **20**). The free amine of the lysine residue side chain is thus beneficial
40 for the activity. However, no dramatic effect of the *N*-terminal free amine of the dipeptide Val-
41 Ala chain was observed in both lysine and valine series. Indeed, a similar activity was obtained
42 for the free amine **3** and the Boc protected **21** from one hand and for the free amine **2** and the
43 Boc protected **20** on the other hand. It was also remarkable that the β anomer **3 β** showed a
44 superior activity to the mixture of α and β anomers in **3** at low compound/A β ₁₋₄₂ ratios (1/1 and
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0.1/1, Table 1 and Figures 2A-C). A supplementary ThT fluorescence assay was performed by first, adding compound **3** after 4 hours, when presumably oligomers are already formed and secondly, adding compound **3** after 42 hours, when presumably essentially fibrils are present (Figure 1S in supporting information). A similar activity was obtained with compound **3** added at the beginning of the kinetics or after 4 hours. However no effect (or even a slightly increase of fluorescence) was observed when compound **3** was added after 42 hours. As also observed in our previous glycopeptides series,^{24b} benzylated derivatives **19**, and **22-23** tended to self-aggregate and to slightly accelerate the aggregation process (Table 3S and Figure 1S in supporting information), confirming that polar hydroxyl groups of the sugar moiety were essential to prevent the aggregation.

Table 1. Effects of compounds **1**, **2**, **3**, **3β**, **20** and **21** on Aβ₁₋₄₂ fibrillization assessed by ThT-fluorescence spectroscopy at a compound/Aβ₁₋₄₂ ratio of 10/1 and 1/1 (the concentration of Aβ₁₋₄₂ in this assay is 10 μM). The effect of **3** and **3β** at a compound/Aβ ratio of 0.1/1 is also reported.

Compounds (Compound/Aβ ratio)	<i>t</i> _{1/2} increase (%) [a]	Plateau decrease (%) [b]
1 10/1	280±70	-56±9
1 1/1	ne	ne
2 10/1	325 ± 12	-31±7
2 1/1	155± 10	ne
3 10/1	NA	-87±1

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2			
3	3 1/1	148±12	-29±9
4			
5	3 0.1/1	ne	-23±6
6			
7			
8			
9	3β 10/1	NA	-90±2
10			
11	3β 1/1	165±11	-34±7
12			
13	3β 0.1/1	129±12	-16±6
14			
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17	20 10/1	379±15	-41±22
18			
19	20 1/1	138±10	ne
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21			
22			
23	21 10/1	NA	-84±3
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25	21 1/1	154±8	-26±6
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ne = no effect, NA = no aggregation, parameters are expressed as mean ± SE, n=3-6. [a] See supporting information for the calculation of the $t_{1/2}$ increase. A compound displaying a $t_{1/2}$ increase > 100 % is a delayer of aggregation. [b] See supporting information for the calculation of the plateau decrease.

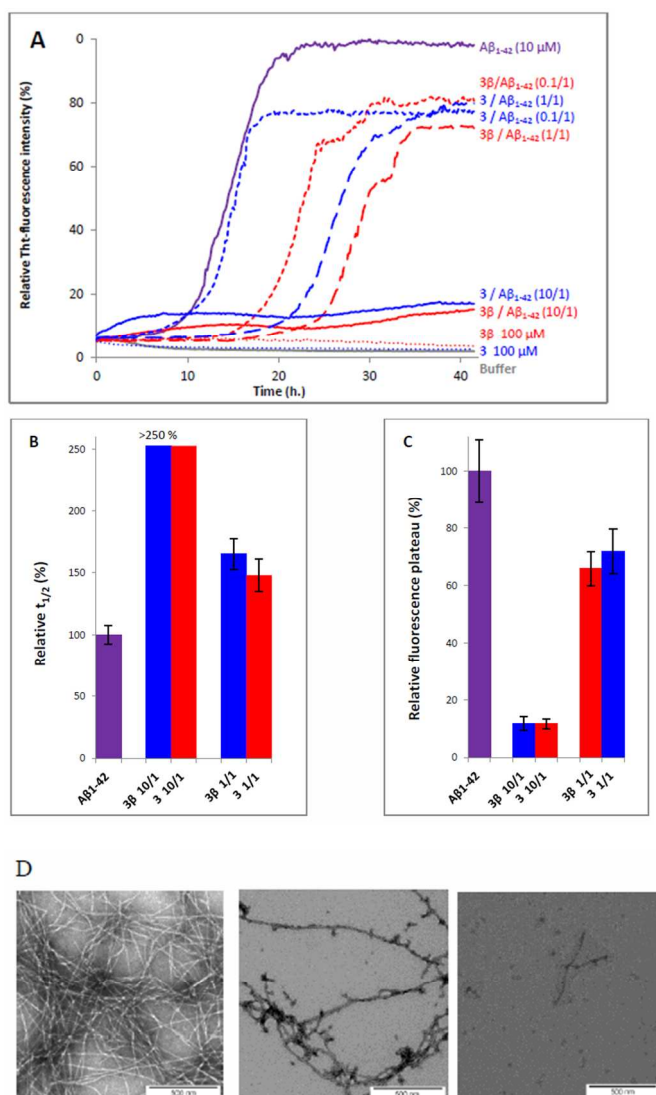


Figure 2. Effects of derivatives **3** and **3 β** on the fibrillization kinetics of $A\beta_{1-42}$ monitored by Thioflavin-T fluorescence and TEM. A) Representative curves of ThT fluorescence assays over time showing $A\beta_{1-42}$ (10 μ M) aggregation in the absence (purple curve) and in the presence of compounds **3** (blue curves) and **3 β** (red curves) at compound/ $A\beta_{1-42}$ ratios of 10/1, 1/1 and 0.1/1. B) $t_{1/2}$ increase relative to $A\beta_{1-42}$ alone, in the presence of compounds **3** (blue curves) and **3 β** (red curves) at compound/ $A\beta_{1-42}$ ratios of 10/1 and 1/1. C) Fluorescence plateau decrease relative to $A\beta_{1-42}$ alone, in the presence of compounds **3** (blue curves) and **3 β** (red curves). D) Effects of derivative **3** on the fibril formation of $A\beta_{1-42}$ visualized by TEM. Negatively stained images were

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3 recorded after 42 h of incubation of A β ₁₋₄₂ (10 μ M in 10 mM Tris.HCl, 100 mM NaCl at pH =
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5 7.4) alone (left) or in the presence of 10 μ M of **3** (middle) and of 100 μ M of **3** (right). Scale bars,
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7 500 nm.
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10 11 *TEM experiments*

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13 Transmission electron microscopy (TEM) analyses were performed on compound **3** that
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15 showed a more significant effect than **2** on A β ₁₋₄₂ aggregation in the ThT-fluorescence assays.
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17 Images were recorded after 42 h of preincubation, corresponding to maximum aggregation in the
18
19 ThT assays, with and without **3** (Figure 2D). Differences were observed regarding the amount of
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21 aggregates formed in the presence of **3** at both ratios. A very dense network of fibers displaying a
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23 typical morphology was observed for A β ₁₋₄₂ alone. Only few scattered, very short and scarce
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25 fibers were visible on the grid containing the A β sample incubated with **3** at 10/1 ratio. This
26
27 result validated the ThT-fluorescence data, indicating that compound **3** dramatically slowed
28
29 down the aggregation of A β ₁₋₄₂ (at **3**/A β ₁₋₄₂ ratio of 10/1) and efficiently reduced the amount of
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31 typical amyloid fibrils formed. It is noteworthy that even if at a **3**/A β ₁₋₄₂ ratio of 1/1 the
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33 fluorescence was not dramatically decreased in the ThT assays, but the morphology of the
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35 network observed by TEM was very different and less dense and the sample contained some
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37 globular aggregates.
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46 **Inhibition of A β ₁₋₄₂ oligomerization by glycopeptidomimetics**

47 48 *Capillary electrophoresis*

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50 In order to determine their effect on small soluble oligomer formation, **3** and **3 β** were studied
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52 by Capillary Electrophoresis (CE). We recently proposed an improved CE method to monitor
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54 easily over time the very early steps of the A β ₁₋₄₂ oligomerization process.^{32,24b} This technique
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3 has the advantage of being able to follow three kinds of soluble species, (i) the monomer (peak
4 ES), (ii) different small metastable oligomers grouped under peak ES' and (iii) transient species
5 formed later and which correspond to species larger than dodecamers (peak LS). Aggregation
6 kinetics of A β ₁₋₄₂ alone showed that over time, the monomer ES peak decreased in favor of the
7 oligomer peaks ES' and LS, and of insoluble species, forming spikes in the profile (Figure 2S in
8 supporting information for the detailed kinetics). At time 0, the monomer peak ES was almost
9 the only visible species, while after 12 h, only a small monomer peak remained and many
10 insoluble aggregates, giving spikes, were present (Figure 3A).

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22 In the presence of **3 β** (**3 β** /A β ₁₋₄₂ ratio of 1/1), the electrophoretic profile clearly indicated that
23 the kinetics of aggregation was significantly slowed down. Indeed, **3 β** maintained dramatically
24 the presence of the monomer (peak ES). In addition, the large oligomer species grouped under
25 the peak LS were still present at 12 h while they completely disappeared in the control
26 electrophoretic profile (Figure 3B, and Figure 3S in supporting information for the detailed
27 kinetics). The preservation of the monomer was statistically significant, after 12 h, only 19%
28 remained in the control experiment while 52% remained in the presence of **3 β** (Figure 3C).
29 Similar results were observed with the mixture of α and β anomers in **3**, however a slightly
30 superior effect was observed for **3 β** (41% of monomer species remained after 12 h in the
31 presence of the mixture **3**) (Figures 4S and 5S in supporting information).
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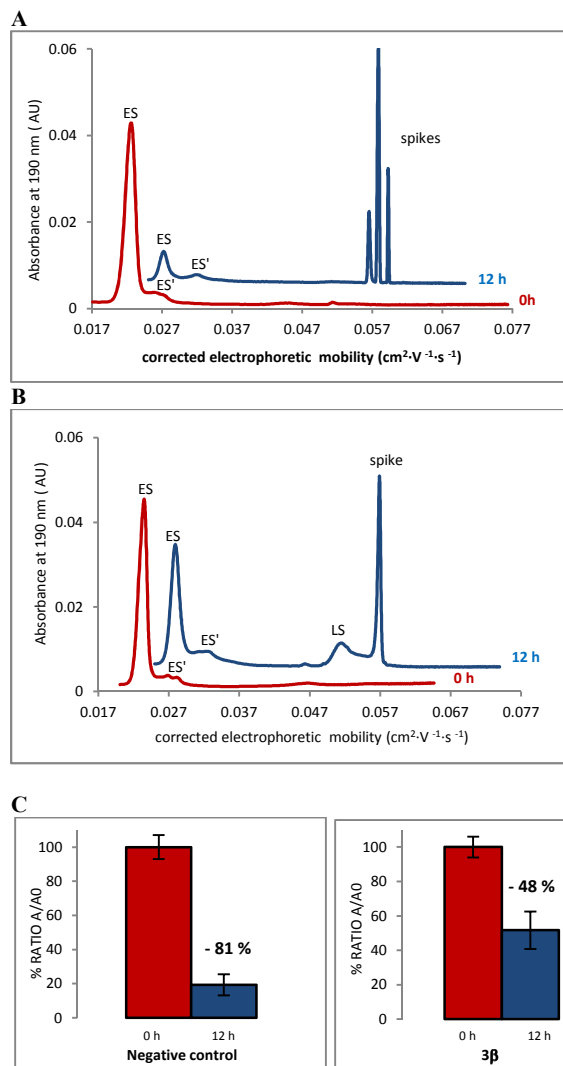


Figure 3. Effect of **3β** on the early oligomerization steps by CE. Electrophoretic profile of $A\beta_{1-42}$ peptide (100 μ M) obtained immediately (0 h), and 12 h after sample reconstitution (t0) alone (A) and in the presence of compound **3β** at compound/ $A\beta_{1-42}$ ratio of 1/1 (B). Results in panel show the effect of **3β** on the monomer ES (C). Results are a mean of 3 experiments.

Interaction of **3β** with monomeric or oligomeric species of $A\beta_{1-42}$

NMR experiments

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3 The goal of the NMR experiments was to study if compound **3β** was able to adopt any
4 preferred conformation in solution and if it interacted in solution either with the monomeric
5 species or with soluble aggregated forms of Aβ₁₋₄₂.
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10 We first examined mixtures of Aβ₁₋₄₂ and **3β** at a temperature of 5°C and using low
11 concentrations of Aβ₁₋₄₂ (10–90 μM) to ensure that Aβ₁₋₄₂ was mainly monomeric in freshly
12 prepared samples.³³ The 2D ¹H-¹⁵N and 2D ¹H-¹³C HSQC spectra of 10 μM ¹⁵N, ¹³C-labelled
13 Aβ₁₋₄₂³⁴ recorded in the absence and in the presence of a large excess of **3β** (0.4 mM) displayed
14 no significant chemical shift perturbations of Aβ₁₋₄₂ ¹H-¹⁵N and ¹H-¹³C correlations (Figure 6S in
15 supporting information). Similarly, no chemical shift differences could be detected for the ¹H
16 signals of **3β** in 1D ¹H and 2D ¹H-¹H experiments (data not shown), even when higher
17 concentrations of Aβ₁₋₄₂ were used (up to 90 μM). Thus NMR experiments demonstrated that **3β**
18 did not interact with monomeric Aβ₁₋₄₂ peptide.
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32 We then turned to magnetization transfer experiments that are commonly used to detect the
33 binding of small ligands to large molecular weight species. Saturation Transfer Difference (STD)
34 experiments were recorded to characterize binding properties and map binding epitopes of **3β**.^{24a,}
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39 ³⁵ No STD signals could be detected in a control experiment with **3β** alone, as expected for a low
40 molecular weight molecule that did not aggregate in solution. The addition of Aβ₁₋₄₂ peptide led
41 to the apparition of weak STD signals (Figure 4). Interestingly, an increase in the STD signal
42 was observed over time, reaching a maximum after 2.5 weeks. Concomitantly, a slow decay of
43 the 1D ¹H NMR signals of Aβ₁₋₄₂ was observed (Figure 7S in supporting information),
44 corresponding to the formation of high molecular weight Aβ₁₋₄₂ aggregates that were too large to
45 be observed by solution NMR spectroscopy.³³ Thus the gradual increase of the STD signal over
46 several weeks could be explained by the slow conversion of monomeric Aβ₁₋₄₂ to aggregated
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3 species that bind **3β**. The STD signals were the strongest for the aromatic and methyl resonances
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5 of **3β**, suggesting that the hydrophobic groups of the dipeptide and peptidomimetic strands were
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7 directly involved in the interaction with Aβ₁₋₄₂ species.
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10 WaterLOGSY experiments also enabled us to detect the binding of **3β** to Aβ₁₋₄₂ species,
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12 through intermolecular magnetization transfers involving bulk water. The protons of **3β**
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14 exhibited positive NOEs in the absence of Aβ₁₋₄₂ (Figure 8S in supporting information), as
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16 expected for a small molecule. The addition of Aβ₁₋₄₂ caused a decrease of positive NOEs and a
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18 change of sign of the NOEs that became more negative over time, confirming that **3β** binds to
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20 high molecular weight species in fast exchange on the NMR time scale.
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24 Finally, NMR spectroscopy was used to analyze the structure of **3β** in the free and bound
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26 forms. The 1D ¹H NMR spectra of **3β** alone were characterized by sharp line widths and
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28 concentration-independent chemical shifts (0.04–2 mM range), demonstrating that **3β** was highly
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30 soluble and not prone to aggregation in the (sub) millimolar range. Chemical shifts, vicinal
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32 coupling constants and ROEs analysis showed that the peptidic/pseudopeptidic arms and the
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34 aminoalkyl and carboxyethyl linkers were highly flexible, as supported by small diastereotopic
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36 splitting of methylenic protons, averaged vicinal coupling constants (Table S1 in supporting
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38 information), intraresidual and sequential ROE intensities, and the absence of long-range ROEs.
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40 Furthermore the amide protons exhibited strong temperature dependence of their chemical shifts
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42 (Table S1 in supporting information), which is an indicator of high solvent accessibility.
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44 Altogether, these NMR data indicated that **3β** did not adopt *per se* hydrogen-bonded β-sheet
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46 conformations and had no self-association properties in solution. Interestingly, 2D NOESY
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48 experiments recorded on **3β** in the presence of Aβ₁₋₄₂ were characterized by modifications in the
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50 intensity of intraresidual and sequential NOEs which became more negative (Figure 9S in
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supporting information). These changes correspond to transferred NOEs due to 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.

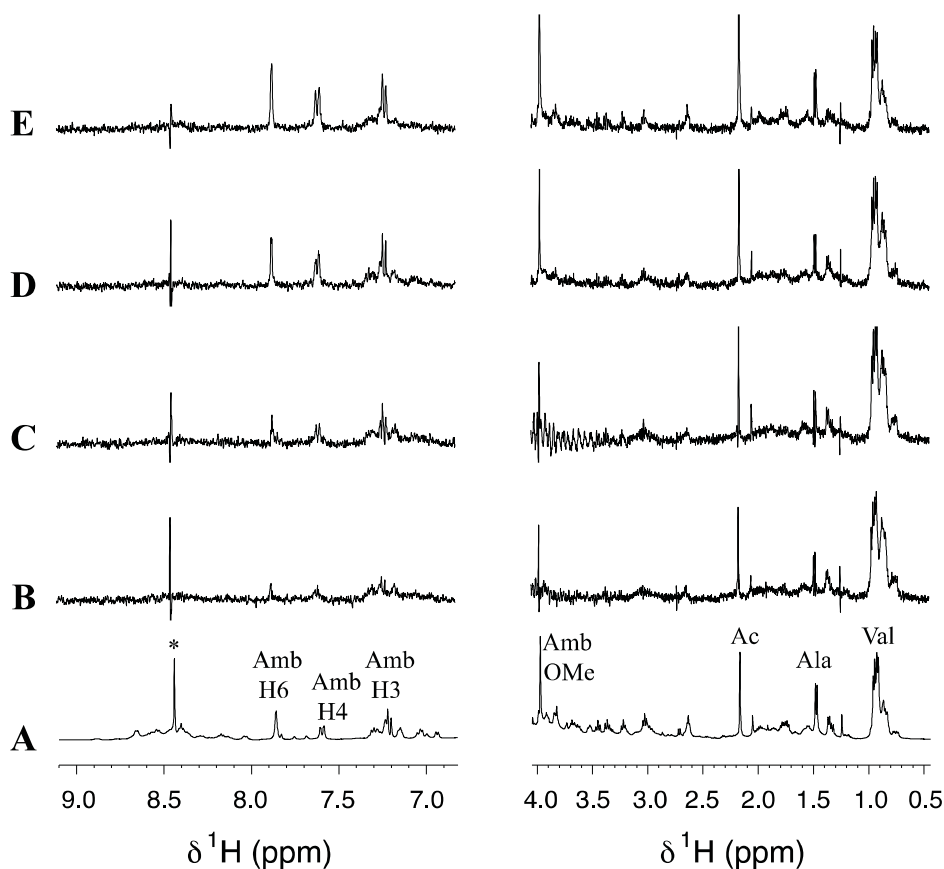


Figure 4. Interaction of **3β** with Aβ₁₋₄₂ monitored by NMR. Aromatic/amide (left) and aliphatic (right) regions of 1D ¹H NMR spectra of **3β** (0.4 mM) and Aβ₁₋₄₂ (90 μM) at 5°C. (A) Reference 1D ¹H spectrum recorded at *t* = 0. (B-E) 1D ¹H STD spectra recorded at *t* = 0 (B), after 2 days (C), 1 week (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.

SPR experiments

SPR was then used to evaluate the affinity between compound **3** and its β -anomer **3 β** and $A\beta_{1-42}$ monomer bound to the gold surface.

To our knowledge, the few SPR experiments described in the literature to detect the affinity of ligands for $A\beta_{1-42}$ have used either the depsipeptide molecule described by Taniguchi et al.^{36,37,22} or biotinylated $A\beta_{1-42}$ immobilized onto streptavidin-coated chips.³⁸ An SPR-based immunoassay has been also developed to recognize $A\beta_{1-42}$ oligomers.³⁹ The main drawbacks we found in these methods are the necessity to synthesize the non-commercial 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 $A\beta_{1-42}$ peptide monomer by a classical peptide coupling through its amino groups. We paid particular attention to maintaining $A\beta_{1-42}$ 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 $A\beta_{1-42}$ 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 $NH_4OH.H_2O$ 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 $A\beta_{1-42}$ (6E10 and MD 19-0016, respectively, see supporting information). Curcumin, which is a well-known disaggregant compound⁴¹ did not lead to a decrease of the signal and was even found to bind to

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3 $A\beta_{1-42}$ fixed on the SPR chips (Figure 18S in supporting information). Finally, the affinity of ThT
4 toward the peptide immobilized on the chip surface was evaluated before and after our optimized
5 rinsing step, which used an aqueous solution of $NH_4OH.H_2O$ 0.1 %. Both SPR signal and
6 fluorescence (visualized by fluorescence microscopy images of the channel) were higher before
7 the rinsing step. The rinsing step is therefore crucial to disaggregate large species present
8 initially on the chip surface in order to lead to a surface mainly composed by $A\beta$ in its
9 monomeric form (Figure 14S in supporting information).

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20 We conducted SPR measurements with compounds **3**, **3b** and **1** to check their affinity for $A\beta_{1-42}$.
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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 $A\beta_{1-42}$ (Figures 15S, 16S and 17S in supporting
information). This result is in accordance with the NMR data.

Protection against $A\beta_{1-42}$ cell toxicity

The inhibitors were investigated to determine their ability to reduce the toxicity of aggregated
 $A\beta_{1-42}$ to SH-SY5Y 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 supporting information) in the presence of cytotoxic 5 μM $A\beta_{1-42}$. Remarkably,
this protective effect was seen at equimolar amounts of inhibitor to $A\beta_{1-42}$ and was still
significant at a very low ratio of 0.1/1 (inhibitor/ $A\beta_{1-42}$) in the MTS assay.

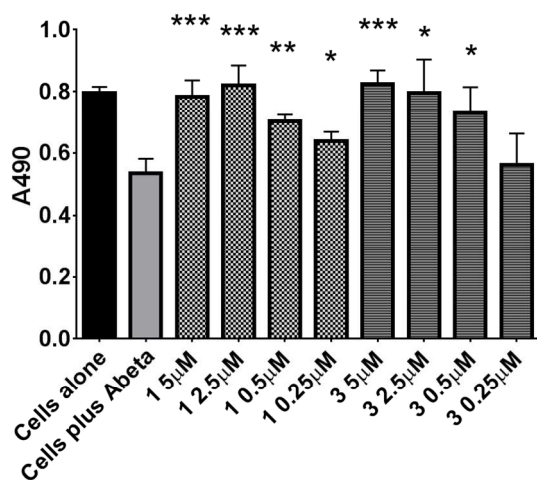


Figure 5. Effect of **1** and **3** on $A\beta_{1-42}$ toxicity towards SH-SY5Y cells. Cell viability in the presence of 5 μM aggregated $A\beta_{1-42}$ and decreasing concentrations of **1** or **3**. The black bar on the left shows cell viability in the absence of $A\beta_{1-42}$. Statistical significance is indicated by *, where * is $p < 0.05$, ** is $p < 0.01$ and *** is $p < 0.001$ comparing cells incubated with $A\beta_{1-42}$ plus inhibitor to those with $A\beta_{1-42}$ alone. Statistical significance is described.

Plasma stability

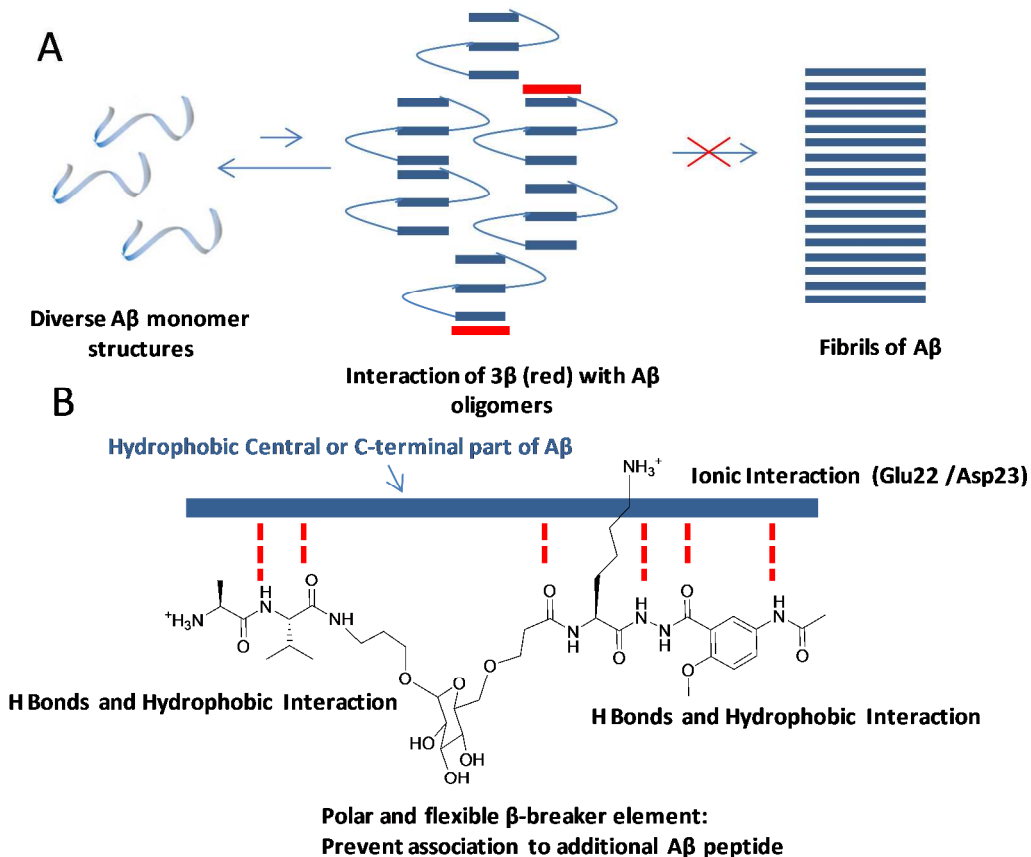
The ability to withstand enzymatic cleavage in the circulatory system is an important requirement for any potential drug. Incubating the two inhibitors **1** and **3** in plasma gives an idea of how stable they will be once injected into the body. **3** withstood 24 hours at 37°C with no obvious degradation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

DISCUSSION

The introduction of a peptidomimetic strand based on a 5-amino-2-methoxybenzhydrazone unit linked through the carboxyethyl in the C6 position of the D-glucopyranosyl scaffold not only increased the stability towards proteolytic degradation but also dramatically increased the capacity of these pentapeptide analogs to inhibit the fibrillization of A β ₁₋₄₂, 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**, **22-23**. A slightly superior effect was observed for the β anomer **3 β** 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 valine residue in compound **2**. This result suggests that an ionic interaction is likely to be established between this amine and acidic residues of A β ₁₋₄₂, strengthening the hydrophobic interactions involving aliphatic and aromatic moieties. Indeed, several computational and experimental studies on A β ₁₋₄₂ 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.^{9,42} 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 A β ₁₋₄₂ (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

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3 oligomerization has been also demonstrated clearly by CE. Compound **3β** dramatically preserved
4 the non-toxic monomer of Aβ₁₋₄₂ (ES). Oligomers larger than dodecamers (LS) were also
5 stabilized. Both types of cell viability assay proved that pre-incubation of cytotoxic Aβ₁₋₄₂ with
6 glycopeptidomimetic **3** completely rescued the SH-SY5Y neuroblastoma cells. The protective
7 effect was observed even at sub-stoichiometric concentrations (**3** reduced cell death by 100%
8 with 0.5 eq and by 75% with 0.1 eq. in the MTS assay). This protective effect is much more
9 pronounced than that observed with molecules which have undergone clinical trials, such as
10 resveratrol⁴³, scyllo-inositol⁴⁴, epigallocatechin-3-gallate (EGCG)^{44,45} or other molecules
11 recently described as efficient reducers of Aβ₁₋₄₂ toxicity.⁴⁶ This effect is comparable to the best
12 effect of the current Aβ aggregation inhibitors reported in the literature.²² Indeed, these
13 molecules reduced Aβ₁₋₄₂ toxicity only at stoichiometric or higher (5 to 10 equivalents)
14 concentrations. It is also noteworthy that glycopeptide **1** showed a dramatic effect on cell
15 survival, but was more sensitive to proteolytic attack.

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

In conclusion, the present work validates the singular effect of sugar-based peptidomimetic analogs of pentapeptides on $A\beta_{1-42}$ 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 $A\beta_{1-42}$, thanks to small peptide and peptidomimetic arms; secondly, to prevent cross β -sheet elongation of $A\beta_{1-42}$ due to the hydrophilic sugar, considered as a β -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 good hydrophobicity/ hydrophilicity balance and resistance to proteolytic degradation. A wide range of bio- and physico-chemical techniques was used to demonstrate the capacity of the compounds (in particular **3 β**) to delay both the early oligomerization and fibrillization of $A\beta_{1-42}$. To the best of our knowledge, this is the first example of a small molecule being able to preserve the non-toxic monomeric species of $A\beta_{1-42}$, as demonstrated by capillary electrophoresis. The strong protective effect on cells, even at sub-stoichiometric concentrations, also highlights the considerable therapeutic potential of this novel series of peptidomimetics. This protective effect is significantly better than the one observed with molecules which have undergone clinical trials. The structural elements demonstrated here as crucial for the inhibitory activity, i.e. hydrophobic moieties, hydrogen bond donors and acceptors, ammonium groups and hydrophilic β -sheet breakers provide valuable insights to explore also the design of compounds targeting other types of amyloid-forming proteins.

EXPERIMENTAL SECTION

Chemistry

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General Experimental Methods. Usual solvents were purchased from commercial sources and dried and distilled by standard procedures. Compounds **4**,²⁹ **10**²⁷ and **11**²⁷ were prepared according to published methods. Pure products were obtained after liquid chromatography using Merck silica gel 60 (40-63 μm). TLC analyses were performed on silica gel 60 F₂₅₀ (0.26 mm thickness) plates. The plates were visualized with UV light ($\lambda = 254 \text{ nm}$) or revealed with a 4 % solution of phosphomolybdic acid in EtOH. Melting points were determined on a Kofler melting point apparatus. Element analyses (C, H, N) were performed on a Perkin-Elmer CHN, Analyser 2400 at the Microanalyses Service of the Faculty of Pharmacy at Châtenay-Malabry (BioCIS, France). NMR spectra were recorded on an ultrafield Bruker AVANCE 300 (¹H, 300 MHz, ¹³C, 75 MHz) or on a Bruker AVANCE 400 (¹H, 400 MHz, ¹³C, 100 MHz). Chemical shifts δ are in ppm and the following abbreviations are used: singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quintuplet (qt), multiplet (m), broad multiplet (bm), and broad singlet (bs). 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**, **19-23** was determined by HPLC using the 1260 Infinity system (Agilent Technologies) and a column SUNFIRE (C18, 3.5 μm , 100 mm X 2.1 mm); mobile phase : MeOH / H₂O + 0.1 % formic acid from 5 to 100 % in 20 min. ; detection at 254 nm ; flow rate 0.25 mL/min. The purity of compounds **3** and **3 β** was determined by HPLC using the 1260 Infinity system (Agilent Technologies) and a column SUNFIRE (C18, 5 μm , 150 mm X 2.1 mm); mobile phase for **3** : acetonitrile / H₂O + 0.2 % formic acid from 1 to 100 % in 20 min. ; mobile phase for **3 β** : acetonitrile / H₂O + 0.2 % formic acid at ratio 1/99 during 3 min., then gradient to 30/70 in 12 min.; detection at 310 nm ; flow rate 0.25 mL/min.

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(2S)-N-[3-[(3S,4S,5S)-6-[[3-[(1S)-1-[(5-Acetamido-2-methoxy-benzoyl)amino]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]methyl]-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxypropyl]-2-[[2S)-2-aminopropanoyl]amino]-3-methylbutanamide (2). Same procedure as described for 16a from 20 (35 mg, 0.036 mmol) in dry CH₂Cl₂ (300 μL) to yield **2** (38 mg, quantitative, α/β 60/40) as a white solid. R_f = 0 (CH₂Cl₂/CH₃OH : 95/5) ; ¹H NMR (300 MHz, CD₃OD) : δ = 7.88 (d, *J* = 2.7 Hz, 1H); 7.53 (dd, *J* = 9.0, 2.7 Hz, 1H); 6.90 (d, *J* = 9.0 Hz, 1H); 4.54 (d, *J* = 3.6 Hz, 0.60H, H_{1β}); 4.19 (d, *J* = 7.0 Hz, 1H); 4.05 (d, *J* = 7.8 Hz, 0.40H, H_{1α}); 3.96 (dd, *J* = 7.7, 2.7 Hz, 1H); 3.86 (m, 1H); 3.76 (s, 3H); 3.64 – 2.96 (m, 12H); 2.36 (m, 2H); 1.96 (m, 1H); 1.91 (s, 3H); 1.81 (m, 1H); 1.58 (m, 2H); 1.29 (d, *J* = 7.0 Hz, 3H); 0.84 (m, 6H); 0.74 (d, *J* = 6.7 Hz, 6H) ; ¹³C NMR (75 MHz, CD₃OD) : δ = 174.2, 174.1, 173.0, 171.6, 171.0, 165.5, 155.6, 133.3, 126.8, 124.3, 120.9, 113.4, 104.3 (C_{1Hα}), 100.2 (C_{1Hβ}), 77.9, 76.6, 75.0, 73.4, 72.5, 71.6, 71.1, 68.5, 68.4, 67.1, 60.7, 58.7, 57.0, 50.1, 37.8, 37.6, 37.3 (C₈), 31.9, 30.0, 23.7, 19.7, 18.7, 17.7; HRMS (TOF, ESI, ion polarity positive, H₂O/MeOH): m/z [M+H]⁺, calcd for C₃₅H₅₈N₇O₁₃ 784.4093; found 784.4094, m/z [M+Na]⁺, calcd for C₃₅H₅₇N₇O₁₃Na 806.3917; found 806.3912; HPLC purity: TR (α, β) = 10.91 min., 95 %.

(2S)-N-[3-[(3S,4S,5S)-6-[[3-[(1S)-1-[(5-Acetamido-2-methoxy-benzoyl)amino]carbamoyl]-5-amino-pentyl]amino]-3-oxo-propoxy]methyl]-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxypropyl]-2-[[2S)-2-aminopropanoyl]amino]-3-methylbutanamide (3). Same procedure as described for 16a from 21 (38 mg, 0.040 mmol) in dry CH₂Cl₂ (300 μL) to afford **3** (40 mg, quantitative, α/β 50/50) as a white solid. R_f = 0 (CH₂Cl₂/CH₃OH : 95/5); ¹H NMR (300 MHz, CD₃OD) : δ = 8.16 (sl, 1H); 7.70 (m, 1H); 7.14 (d, *J* = 9.0 Hz, 1H); 4.75 (m, 0.50H, H_{1β}); 4.54 (m, 1H); 4.26 (d, *J* = 7.8 Hz, 0.50H, H_{1α}); 4.14 (m, 1H); 4.02 (d, *J* = 7.1 Hz, 1H); 3.98 (s, 3H); 3.90 (m, 1H); 3.79 (m, 3H); 3.70 (m, 1H); 3.62 (m, 2H); 3.35

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3 (m, 4.6H); 3.16 (m, 0.6H); 3.04 (m, 1H); 2.98 (m, 1H); 2.55 (sl, 2H); 2.12 (s, 3H); 2.03 (m, 1H);
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5 1.90 (m, 2H); 1.77 (m, 4H); 1.57 (m, 2H); 1.50 (d, $J = 7.1$ Hz, 3H); 1.34 (d, $J = 6.5$ Hz, 4H);
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7 0.97 (d, $J = 6.5$ Hz, 6H) ; ^{13}C NMR (125 MHz, $\text{H}_2\text{O}:\text{D}_2\text{O}$ 90:10) : $\delta = 177.5, 177.1, 175.9,$
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9 175.6, 173.6, 169.5, 169.2, 165.6, 158.1, 132.8, 131.5, 131.4, 130.18, 127.8, 121.4, ,121.2, 120.1,
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11 117.8, 115.6, 104.9 ($\text{C}_{1\text{H}\alpha}$), 103.0, 100.9 ($\text{C}_{1\text{H}\beta}$), 78.3, 77.3, 75.8, 73.1, 72.4, 72.1, 71.5, 70.3,
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13 69.9, 69.8, 68.1, 63.1, 58.8, 55.6, 55.27, 53.5, 51.6, 47.3, 42.1, 42.0, 39.2, 39.0, 38.5, 38.3, 34.1,
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15 33.2, 33.1, 32.6, 31.1, 31.0, 29.2, 29.1, 28.2, 25.3, 24.9, 24.7, 21.1, 21.0, 20.8, 19.5 ; HRMS
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17 (TOF, ESI, ion polarity positive, $\text{H}_2\text{O}/\text{MeOH}$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{36}\text{H}_{61}\text{N}_8\text{O}_{13}$ 813.4358;
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19 found 813.4363; HPLC purity: TR (α, β) = 11.70 min., 100 %.

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25 **(2S)-N-[3-[(2R,3S,4S,5S)-6-[[3-[(1S)-1-[(5-Acetamido-2-methoxy-**

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27 **benzoyl)amino]carbamoyle]-5-amino-pentyl]amino]-3-oxo-propoxy)methyl]-3,4,5-**

28
29 **trihydroxy-tetrahydropyran-2-yl]oxypropyl]-2-[[2S)-2-aminopropanoyl]amino]-3-methyl-**

30
31 **butanamide (3 β).** Isolation of β anomer **3 β** from **3** was performed by HPLC using a WATERS
32
33 gradient system pump (DELTAPREP, UV detector PDA 2996) and a column sunfire (C18, 5
34
35 μm , 150 mm x 19 mm); mobile phase: acetonitrile / H_2O + 0.2 % formic acid at ratio 1/99 during
36
37 3 min., then at ratio 60/40 in 12 min. ; flow rate 17 mL/min. ; detection at 310 nm. HRMS
38
39 (TOF, ESI, ion polarity positive, $\text{H}_2\text{O}/\text{MeOH}$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{36}\text{H}_{61}\text{N}_8\text{O}_{13}$ 813.4358;
40
41 found 813.4356 ; HPLC purity: TR = 10.12 min., 100 %. ^1H and ^{13}C NMR assignments are
42
43 shown in supporting information (Tables S1 and S2)

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47
48 **tert-Butyl 3-[(6-allyloxy-3,4,5-tribenzyloxy-tetrahydropyran-2-yl)methoxy]propanoate**
49
50 **(5).** To a suspension of **4** (7.00 g, 14.27 mmol) in *tert*-butyl-acrylate (4.14 mL, 28.54 mmol) was
51
52 added TBAB (690 mg, 2.14 mmol) and an aqueous solution of NaOH 20% (60 mL). The
53
54 reaction mixture, which appeared like an emulsion, was stirred for 24 h at room temperature. At
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3 the end of the reaction, a mixture of EtOAc/H₂O 1/1 (200 mL) was added and the aqueous phase
4
5 was extracted with EtOAc (3 x 60 mL). The combined organic layers were dried over Na₂SO₄,
6
7 filtered and concentrated under reduced pressure to give a crude oil which was purified by
8
9 column chromatography on silica gel with eluent cyclohexane/EtOAc 80/20 to afford **5** as a
10
11 colourless oil (7.1 g, 79%, α/β 70/30). $R_f = 0.45$ (cyclohexane/EtOAc : 80/20); ¹H NMR (300
12
13 MHz, CDCl₃) : $\delta = 7.34$ (m, 15H); 5.93 (m, 1H); 5.32 (dd, $J = 17.2, 1.5$ Hz, 1H); 5.22 (d, $J =$
14
15 10.0 Hz, 1H); 4.86 (d, $J = 4.9$ Hz, 0.75H, H_{1 β}); 4.45 (d, $J = 7.8$ Hz, 0.25H, H_{1 α}); 5.10-4.54 (m,
16
17 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
18
19 = 170.7, 138.9, 138.4, 138.2, 133.8, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7, 127.5,
20
21 118.2, 95.7, 82.1, 80.5, 79.9, 77.6, 75.7, 75.0, 73.2, 70.2, 69.5, 68.2, 67.1, 36.1, 28.1; Anal.
22
23 Calcd for C₃₇H₄₆O₈: C, 71.82; H, 7.49; Found C, 71.75; H, 7.65; MS (ESI, ion polarity positive,
24
25 MeOH) : m/z: 641.4 [M+Na]⁺.
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32 **tert-Butyl 3-[(3,4,5-tribenzyloxy-6-hydroxy-tetrahydropyran-2-yl)methoxy]propanoate**

33
34 **(6)**. To a stirred mixture of **5** (7.10 g, 11.47 mmol) in CH₃OH /EtOH 2/1 (60 mL) was added
35
36 PdCl₂ (170 mg, mass 2.4%) at room temperature. The brown suspension was stirred under azote
37
38 atmosphere overnight and became darker and finally black. The reaction mixture was filtered
39
40 through a pad of Celite which was washed several times with CH₃OH. The filtrate was then
41
42 concentrated under reduced pressure to obtain a brown oil, which was purified by column
43
44 chromatography on silica gel with eluent cyclohexane/EtOAc 90/10 to yield **6** as a yellow oil
45
46 (4.61 g, 75%, α/β 70/30). $R_f = 0.15$ (cyclohexane/EtOAc : 90/10) ; ¹H NMR (300 MHz, CDCl₃) :
47
48 $\delta = 7.32$ (m, 15H); 5.32 (s, 0.70H, H_{1 β}); 5.04- 4.56 (m, 6.30H, 6H+H_{1 α}); 4.22-3.36 (m, 8H); 2.52
49
50 (t, $J = 6.5$ Hz, 2H); 1.45 (s, 9H) ; ¹³C NMR (75 MHz, CDCl₃) : $\delta = 170.7, 138.9, 138.4, 138.2,$
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3 128.5, 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,
4
5 73.2, 70.2, 69.5, 67.1, 36.1, 28.1; MS (ESI, ion polarity positive, MeOH) : m/z: 601.4 [M+Na]⁺.
6
7

8 **tert-Butyl 3-[[3,4,5-tribenzyloxy-6-(2,2,2-trichloroethan imidoyl)oxy-tetrahydropyran-2-**
9 **yl]methoxy] propanoate (7).** To a solution of **6** (4.61 g, 7.96 mmol) in dry CH₂Cl₂ (80 mL)
10 cooled at 0°C was gradually added under azote atmosphere NaH (60% dispersion in mineral oil,
11 180 mg; 4.50 mmol). Then trichloroacetonitrile (3.8 mL, 38.06 mmol, 11 eq.) was added. After
12 20 minutes, the ice bath was removed and the reaction mixture was let to stir at room
13 temperature for 4 hours. The solution turned from yellowish to orange while stirring and finally
14 became brown. After stirring overnight, the solvent was evaporated under reduced pressure and
15 the crude residue was purified by column chromatography on neutral alumina with eluent
16 cyclohexane/EtOAc 80/20 to give **7** as a yellow oil (4.31 g, 75%, α/β 85/15). $R_f = 0.45$
17 (cyclohexane/EtOAc : 80/20) ; ¹H NMR (300 MHz, CDCl₃) : $\delta = 8.59$ (s, 1H); 7.31 (m, 15H);
18 6.53 (d, $J = 3.4$ Hz, 0.84H, H_{1 β}); 5.03-4.61 (m, 6.16H, 6H+H_{1 α}); 4.27-3.48 (m, 8H); 2.51 (t, $J =$
19 6.6 Hz, 2H); 1.44 (s, 9H).
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36 **tert-Butyl 3-(((6-(3-azidopropoxy)-3,4,5-tris(benzyloxy)-tetrahydropyran-2-yl)methoxy)**
37 **propanoate (8).** To a stirred solution of **7** (4.31 g, 5.96 mmol) and 3-azidopropan-1-ol (723 mg,
38 7.15 mmol) in dry CH₂Cl₂ (15 mL) under azote atmosphere, was added AuCl (181 mg, mass
39 10%). The reaction mixture was stirred for 2 days at room temperature under azote atmosphere.
40 Upon completion of the reaction monitored by TLC, the mixture was filtered to remove the
41 catalyst and the filtrate was concentrated under reduced pressure. The oily residue afforded was
42 purified by column chromatography on silica gel with eluent cyclohexane/EtOAc 80/20 to give **8**
43 as a yellow oil (3.25 g, 82%, α/β 50/50). $R_f = 0.45$ (cyclohexane/EtOAc : 80/20) ; ¹H NMR (300
44 MHz, CDCl₃) : $\delta = 7.33$ (m, 15H); 5.08-4.55 (m, 6.50H, 6H+H_{1 β}); 4.38 (d, $J = 7.8$ Hz, 0.50H,
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3 H_{1α}); 4.13-3.23 (m, 12H); 2.51 (t, *J* = 6.6 Hz, 2H); 1.91 (m, 2H); 1.45 (s, 9H) ; ¹³C NMR (75
4
5 MHz, CDCl₃) : δ = 170.8, 138.9, 138.6, 138.4, 138.2, 128.4, 128.3, 128.0, 127.8, 127.6, 103.6
6
7 (C_{1Hα}), 97.2 (C_{1Hβ}), 84.7, 82.3, 82.0, 80.5, 80.1, 77.8, 76.6, 75.6, 75.1, 74.9, 73.3, 70.4, 69.8,
8
9 69.5, 67.3, 66.6, 64.7, 48.4, 36.3, 36.1, 29.3, 28.9, 28.1, 27.0 ; IR (neat) : 2096 (N₃); 1728
10
11 (C=O); 1366 (CH₃); 1066 (C-O) cm⁻¹ ; Anal. Calcd for C₃₇H₄₇N₃O₈ · 0.5 H₂O : C, 66.25; H, 7.23;
12
13 N, 6.27 Found C, 66.11; H, 7.04; N, 5.81 ; MS (ESI, ion polarity positive, MeOH) : m/z: 684.7
14
15 [M+Na]⁺.
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18

19
20 **3-((6-(3-Azidopropoxy)-3,4,5-tris(benzyloxy)-tetrahydropyran-2-yl)methoxy)propanoic**
21
22 **acid (9)**. To a stirred solution of **8** (3.21 g, 4.85 mmol) in dry CH₂Cl₂ (40 mL) cooled at 0°C was
23
24 cautiously added TFA (18.6 mL, 250.40 mmol). The reaction mixture appeared like a yellow
25
26 solution and was stirred overnight at room temperature. The day after, the solvent was
27
28 evaporated under reduced pressure and the crude residue was purified by column
29
30 chromatography on silica gel beginning with CH₂Cl₂ 100% as eluent and finishing with an eluent
31
32 mixture CH₂Cl₂/CH₃OH 95/5 to provide **9** as a yellow oil (2.11 g, 72%, α/β 50/50). R_f = 0.40
33
34 (CH₂Cl₂/CH₃OH 95/5) ; ¹H NMR (300 MHz, CDCl₃) : δ = 7.32 (m, 15H); 6.93 (s, 1H); 5.04-
35
36 4.54 (m, 6.50H, 6H+H_{1β}); 4.40 (d, *J* = 7.8 Hz, 0.50H, H_{1α}); 4.08-3.32 (m, 12H); 2.63 (m, 2H);
37
38 2.03-1.71 (m, 2H) ; ¹³C NMR (75 MHz, CDCl₃) : δ = 175.4, 138.8, 138.5, 138.4, 138.2, 138.1,
39
40 128.4, 128.0, 127.9, 127.6, 103.6 (C_{1Hα}), 97.2 (C_{1Hβ}), 84.6, 82.2, 82.0, 80.1, 75.7, 75.1, 74.9,
41
42 74.6, 73.3, 70.2, 69.9, 69.7, 66.6, 64.8, 48.3, 34.7, 34.5, 29.3, 28.9; IR (neat) : 2097 (N₃); 1714
43
44 (C=O); 1065 (C-O) cm⁻¹; MS (ESI, ion polarity positive, MeOH) : m/z: 629 [M+Na]⁺.
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52 **N-[(1S)-1-[[5-Acetamido-2-methoxy-benzoyl]amino]carbonyl]-2-methyl-propyl]-3-**
53
54 **[[3S,4S,5S)-3,4,5-tribenzyloxy-6-[3-[(imino-5-azanylidene)amino]propoxy]**
55
56 **tetrahydropyran-2-yl]methoxy]propanamide (12)**. To a stirred solution of **10** (360 mg, 0.83
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3 mmol) in dry DMF (5 mL) and cooled at 0°C under azote atmosphere, were successively added
4
5 DMTMM (270 mg, 0.83 mmol) and NMM (272 μ L, 2.48 mmol). The reaction mixture was
6
7 stirred at 0°C for 1h. Then, 9 (500 mg, 0.83 mmol) in dry DMF (5 mL) was added to the reaction
8
9 mixture. The ice bath was removed after 30 minutes and the orange reaction mixture was stirred
10
11 at room temperature overnight. The solvent was evaporated under reduced pressure and the oily
12
13 residue was taken up with EtOAc (50 mL). The organic layer was successively washed with
14
15 distilled water, 10% citric acid aqueous solution, 10% aqueous K₂CO₃ solution and brine, dried
16
17 over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The yellow crude
18
19 product was purified by puriflash column chromatography (SI-HP, 12 g, 22 bars, 30 μ m, flow
20
21 rate: 20 mL/min) with eluent CH₂Cl₂/CH₃OH 95/5 to afford **12** as a white solid (593 mg, 86%,
22
23 α/β 40/60); mp = 71-73 °C ; R_f = 0.60 (CH₂Cl₂/CH₃OH 95/5) ; ¹H NMR (300 MHz, CDCl₃) : δ
24
25 = 12.05 (t, *J* = 6.2 Hz, 1H, NH); 11.28 (d, *J* = 6.3 Hz, 1H, NH); 9.80 (s, 1H); 8.58 (dd, *J* = 9.1,
26
27 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-
28
29 4.58 (m, 6.40H, 6H+H_{1 β}); 4.40 (d, *J* = 7.8 Hz, 0.60H, H_{1 α}); 4.02-3.44 (m, 15H); 2.59 (m, 2H);
30
31 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,
32
33 CDCl₃) : δ = 171.2, 171.1, 169.0, 164.9, 158.2, 158.1, 153.1, 138.9, 138.6, 138.4, 138.3, 138.2,
34
35 138.1, 133.9, 128.4, 128.3, 128.0, 127.9, 127.8, 127.5, 124.7, 122.5, 117.7, 111.8, 103.6 (C_{1H α}),
36
37 97.1 (C_{1H β}), 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,
38
39 67.6, 66.6, 64.8, 56.4, 55.8, 48.3, 37.6, 37.5, 33.4, 33.3, 29.3, 28.9, 24.3, 18.9, 18.2 ; IR (neat) :
40
41 3375 (N-H); 2097 (N₃) cm⁻¹; Anal. Calcd for C₄₈H₅₉N₇O₁₁ · 0.5 H₂O : C, 62.73; H, 6.58; N, 10.67
42
43 Found C, 62.78; H, 6.98; N, 10.55 ; MS (ESI, ion polarity negative, MeOH) : m/z: 908.0
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45 [M-H]⁻.
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3 **Benzyl N-[(5S)-6-[2-(5-acetamido-2-methoxy-benzoyl)hydrazino]-6-oxo-5-[3-[[[(3S,4S,5S)-**
4 **3,4,5-tribenzyloxy-6-[3-[(imino-5-azanylidene)amino]propoxy]tetrahydropyran-2-**
5 **yl]methoxy] propanoylamino]hexyl]carbamate (13).** Same procedure as described for **12** from
6
7
8 **11b** (495 mg, 0.826 mmol) and **9** (500 mg, 0.826 mmol). The yellow crude product obtained was
9
10 purified by puriflash column chromatography (SI-HP, 12 g, 22bar, 30 μ m, flow rate: 20 mL/min)
11
12 with eluent CH₂Cl₂/CH₃OH 95/5 to afford **13** as a white solid (593 mg, 68%, α/β 50/50). R_f =
13
14 0.15 et 0.25 (two diastereoisomers) (CH₂Cl₂/CH₃OH 95/5) ; ¹H NMR (300 MHz, CDCl₃) : δ =
15
16 11.97 (sl, 1H, NH) ; 11.32 (sl, 1H, NH); 9.80 (sl, 1H, NH); 8.58 (d, *J* = 7.7 Hz, 1H); 8.17 (s,
17
18 1H); 7.32 - 7.26 (m, 21H); 6.91 (m, 1H); 5.41 (m, 1H); 5.07 – 4.60 (m, 9.50H, 9H+H_{1 β}); 4.42 (d,
19
20 *J* = 7.8 Hz, 0.50H, H_{1 α}); 4.11 – 3.32 (m, 15H); 2.99 (m, 2H); 2.59 (s, 2H); 2.21 (s, 3H); 1.88 (m,
21
22 2H); 1.37 (m, 6H) ; ¹³C NMR (75 MHz, CDCl₃) : δ = 174.2, 173.3, 169.2, 165.5, 156.3, 153.3,
23
24 138.5, 138.0, 137.8, 136.7, 133.8, 128.5, 128.3, 128.0, 127.8, 125.0, 122.1 (C₃₀), 117.7, 111.9,
25
26 103.6 (C_{1H α}), 97.2 (C_{1H β}), 92.2, 87.2, 84.6, 82.0, 81.7, 75.6, 66.7 (C₁₆), 56.4, 50.8, 48.3, 40.6,
27
28 37.6, 34.1, 34.0, 33.9, 29.1, 24.0, 22.2, 20.48; MS (ESI, ion polarity positive, MeOH) : m/z:
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30 1095.48 [M+Na]⁺.
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39 **N-[(1S)-1-[[[(5-Acetamido-2-methoxy-benzoyl)amino]carbamoyl]-2-methyl-propyl]-3-**
40 **[[[(3S,4S,5S)-6-(3-aminopropoxy)-3,4,5-tribenzyloxy-tetrahydropyran-2-**
41 **yl]methoxy]propanamide (14).** To a stirred solution of **12** (174 mg, 0.19 mmol) in THF (2 mL)
42
43 was added at room temperature Ph₃P (100 mg, 0.38 mmol). After 10 minutes, H₂O (0.3 mL) was
44
45 added and the reaction was stirred at reflux at 40°C for 24 h. The solvents were evaporated under
46
47 reduced pressure and the oily residue obtained was purified by column chromatography on silica
48
49 gel successively eluting with CH₂Cl₂ 100%, then CH₂Cl₂/CH₃OH 95/5, CH₂Cl₂/CH₃OH 90/10
50
51 to eliminate the impurities and finally with CH₂Cl₂/CH₃OH/ NH₄OH (20% aq. sol.) 88/9/3 to
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3 afford **14** (107 mg, 63%, α/β 40/60) as a white solid; mp = 203-205 °C ; R_f = 0.10
4
5 (CH₂Cl₂/CH₃OH/NH₄OH (20% aq. sol.) 88/9/3 ; ¹H NMR (300 MHz, CDCl₃) : δ = 9.71 (bs,
6
7 1H); 8.55 (dd, J = 9.1, 2.7 Hz, 1H); 8.25 (d, J = 2.7 Hz, 1H); 7.31-7.20 (m, 16H); 6.92 (d, J =
8
9 9.1, 1H); 5.25 (m, 1H); 4.89-4.57 (m, 6,40 H, 6H+H_{1 β}); 4.41 (d, J = 7.8 Hz, 0.60H, H_{1 α}); 3.94 (s,
10
11 3H); 3.91 – 3.36 (m, 12H); 2.85 (m, 2H); 2.58 (m, 2H); 2.17 (s, 3H); 2.08 (m, 1H); 1.78 (m, 2H);
12
13 0.91 (m, 6H) ; ¹³C NMR (75 MHz, CDCl₃) : δ = 171.3, 169.0, 165.4, 158.4, 153.1, 138.6, 138.5,
14
15 138.0, 133.9, 128.4, 128.3, 128.0, 127.8, 127.6, 127.5, 124.8, 122.5, 117.8, 111.8, 103.6, 84.6,
16
17 82.3, 77.9, 75.5, 75.0, 74.8, 70.2, 67.7, 56.4, 56.0, 38.9, 37.5, 33.2, 33.0, 24.3, 19.0, 18.3 ; IR
18
19 (neat) : 3465, 3418 (HN-H) cm⁻¹; Anal. Calcd for C₄₈H₆₁N₅O₁₁ · (CH₃)₂CO : C, 65.02; H, 7.18; N,
20
21 7.44 Found C, 64.88; H, 7.55; N, 7.16 ; MS (ESI, ion polarity negative, MeOH) : m/z: 882.7
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23 [M-H]⁻.
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30 **Benzyl N-[(5S)-6-[2-(5-acetamido-2-methoxy-benzoyl)hydrazino]-5-[3-[[3S,4S,5S)-6-(3-**
31 **aminopropoxy)-3,4,5-tribenzyloxy-tetrahydropyran-2-yl]methoxy]propanoylamino]-6-oxo-**
32 **hexyl]carbamate (15).** Same procedure as described for **14** from **13** (497 mg, 0.463 mmol) to
33
34 afford **15** (247 mg, 50%, α/β 50/50) as a white solid. R_f = 0.1 (CH₂Cl₂/CH₃OH/NH₄OH (20% aq.
35
36 sol.) 88/9/3) ; ¹H NMR (300 MHz, CD₃OD) : δ = 8.08 (dd, J = 12.4, 2.7 Hz, 1H); 7.70 (m, 1H);
37
38 7.34-7.22 (m, 20H); 6.98 (m, 1H); 4.98 (s, 2H); 4.97 – 4.53 (m, 8.50H, 8H+H_{1 β}); 4.41 (m, 1H),
39
40 4.37 (d, J = 7.7 Hz, 0.50H, H_{1 α}); 3.86 (s, 3H), 3.80 – 3.21 (m, 10H) 3.04 (m, 2H); 2.85 (m, 2H)
41
42 2.48 (m, 2H); 2.03 (m, 3H); 1.87 – 1.67 (m, 4H); 1.42 (m, 4H) ; ¹³C NMR (75 MHz, CD₃OD) : δ
43
44 = 173.9, 171.5, 171.3, 164.6, 164.2, 158.8, 155.5, 140.0, 139.9, 139.6, 139.5, 138.3, 133.5,
45
46 129.4-128.6, 126.6, 126.3, 124.3, 124.2, 121.3, 121.2, 113.4, 104.7 (C_{1H α}), 98.0 (C_{1H β}), 85.6,
47
48 83.4, 83.0, 81.4, 79.1, 78.9, 76.4, 76.0, 75.9, 75.6, 75.3, 74.0, 71.7, 70.7, 68.6, 68.5, 68.3, 67.5,
49
50 67.3, 56.9, 53.8, 53.7, 49.9, 41.5, 39.8, 39.3, 37.5, 37.3, 33.0, 32.9, 30.9, 30.4, 24.0, 23.9, 23.7 ;
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3 HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+H]⁺, calcd for C₅₇H₇₁N₆O₁₃
4
5 1047.5079; found 1047.5063.
6
7

8 **tert-Butyl N-[(1*S*)-1-[3-[6-[[3-[[**(1*S*)-1-[(5-acetamido-2-methoxy-benzoyl)amino]**
9 **carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]methyl]-3,4,5-tribenzyloxy-**
10 **tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methyl-propyl]carbamate (16).** To a stirred
11 solution of *N*-Boc-Val-OH (132 mg, 0.60 mmol) in dry DMF (2 mL) and cooled at 0 °C, under
12 azote atmosphere, were successively added DMTMM (98.4 mg, 0.30 mmol) and NMM (100 μL,
13 0.91 mmol). The reaction mixture was stirred at 0 °C for 1h. Then **14** (270 mg, 0.30 mmol) in
14 dry DMF (2 mL) was added to the reaction mixture. The ice bath was removed after 30 minutes
15 and the orange reaction mixture was stirred at room temperature overnight. The solvent was
16 evaporated under reduced pressure and the oily residue obtained was taken up with EtOAc (50
17 mL). The organic layer was successively washed with distilled water, 10% citric acid aqueous
18 solution, 10% K₂CO₃ aqueous solution, and brine, dried over anhydrous Na₂SO₄, filtered and
19 concentrated under reduced pressure. The oily crude product was purified by puriflash column
20 chromatography (SI-HP, 12 g, 22 bars, 30 μm, flow rate: 20 mL/min) with eluent
21 CH₂Cl₂/CH₃OH 95/5 to afford **16** (260 mg, 79%, α/β 40/60) as a white solid. R_f = 0.35
22 (CH₂Cl₂/CH₃OH 95/5); ¹H NMR (300 MHz, CD₃OD): δ = 8.04 (s, 1H); 7.80 (d, *J* = 8.8, 2.7 Hz,
23 1H); 7.34-7.25 (m, 15H); 7.10 (d, *J* = 8.8 Hz, 1H); 4.93-4.61 (m, 6.40H, 6H+H_{1β}); 4.44 (d, *J* =
24 7.8 Hz, 0.60H, H_{1α}); 4.34 (d, *J* = 6.8 Hz, 1H); 3.94 (s, 3H); 3.81-3.29 (m, 13H); 2.61-2.46 (m,
25 2H); 2.14-2.09 (m, 4H); 1.99-1.79 (m, 3H); 1.45 (s, 9H); 1.04 (dd, *J* = 11.2, 6.7 Hz, 6H); 0.93 (m,
26 6H); ¹³C NMR (75 MHz, CD₃OD): δ = 174.4, 174.2, 171.5, 171.4, 157.9, 155.6, 140.3, 139.9,
27 139.6, 133.5, 129.5, 129.3, 129.0, 128.9, 128.6, 128.5, 126.7, 124.3, 121.2, 113.4, 104.8 (C_{1Hα}),
28 98.1 (C_{1Hβ}), 83.0, 81.4, 78.9, 76.4, 76.0, 75.9, 75.8, 73.9, 71.6, 70.5, 68.6, 67.4, 61.8, 58.9, 57.0,
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38.0, 37.5, 32.0, 31.6, 30.3, 28.7, 23.6, 19.8, 18.9, 18.6; MS (ESI, ion polarity negative, MeOH) :
m/z: 1081.9 [M-H]⁻.

**tert-Butyl N-[(1S)-2-[[[(1S)-1-[3-[(3S,4S,5R)-6-[[3-[[[(1S)-1-[[5-acetamido-2-methoxy-
benzoyl]amino]carbamoyl]-5-(benzyloxycarbonylamino)pentyl]amino]-3-oxo-
propoxy]methyl]-3,4,5-tribenzyloxy-tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methyl-
propyl]amino]-1-methyl-2-oxo-ethyl]carbamate (17).** Same procedure as described for **16**
from **15** (245 mg, 0.234 mmol) to afford **17** as a white solid (200 mg, 68%, α/β 50/50). R_f = 0.35
et 0.40 (two diastereoisomers) (CH₂Cl₂/CH₃OH : 95/5) ; ¹H NMR (300 MHz, CD₃OD) : δ = 8.06
(s, 1H) ; 7.83 (m, 1H); 7.29-7.23 (m, 20H); 7.04 (dd, J = 9.0, 3.6 Hz, 1H); 5.03 (m, 2H); 4.91 –
4.57 (m, 6.50H, 6H+H_{1 β}); 4.51 (m, 1H); 4.40 (d, J = 7.8 Hz, 0.50H, H_{1 α}); 3.88 (s, 3H); 3.84 –
3.48 (m, 10H); 3.43-3.28 (m, 3H); 3.08 (m, 2H); 2.53 (m, 2H); 2.08 (m, 3H); 2.00 – 1.78 (m,
5H); 1.49 (m, 4H); 1.42 (s, 9H); 0.90 (m, 6H) ; ¹³C NMR (75 MHz, CD₃OD) : δ = 174.3, 174.1,
174.0, 172.1, 171.4, 165.3, 158.8, 157.8, 155.5, 140-139.6, 138.4, 133.6, 129.4- 128.5, 126.8,
124.3, 120.9, 113.4, 104.8 (C_{1H α}), 98.1 (C_{1H β}), 85.7, 83.5, 83.0, 81.6, 80.5, 79.1, 76.5, 76.0, 75.7,
73.9, 71.6, 70.8, 70.6, 68.5, 67.3, 61.7, 61.6, 57.0, 53.3, 41.5, 37.6, 32.8, 32.7, 32.1, 30.7, 30.4,
28.7, 24.0, 23.9, 23.7, 19.8, 18.6 ; MS (ESI, ion polarity positive, MeOH) : m/z: 1268.61
[M+Na]⁺. HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+Na]⁺, calcd for
C₆₇H₈₇N₇O₁₆Na 1268.6102; found 1268.6093.

**tert-Butyl N-[(1S)-2-[[[(1S)-1-[3-[6-[[3-[[[(1S)-1-[[5-acetamido-2-methoxy-
benzoyl]amino]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]methyl]-3,4,5-
tribenzyloxy-tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methyl-propyl]amino]-1-
methyl-2-oxo-ethyl]carbamate (18).** To a stirred solution of **16** (270 mg, 0.25 mmol) in dry
CH₂Cl₂ (2 mL), cooled to 0 °C was carefully added TFA (1 mL). After 30 minutes the ice bath

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2
3 was removed and the reaction mixture was stirred at room temperature for 3 h. The solvent was
4
5 evaporated at reduced pressure and the viscous oily residue was taken up with toluene and again
6
7 evaporated. The oily residue obtained was triturated in Et₂O to yield the TFA salt of the free
8
9 amine **16a** (301 mg, quantitative, α/β 50/50) as a white solid. R_f = 0 (CH₂Cl₂/CH₃OH : 95/5) ; ¹H
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11 NMR (300 MHz, CD₃OD) : δ = 8.06 (m, 1H); 7.72 (dd, *J* = 8.7, 2.8 Hz, 1H); 7.28-7.21 (m,
12
13 15H); 7.03 (dd, *J* = 9.0, 2.3 Hz, 1H); 4.86-4.56 (m, 6.50H, 6H+H_{1β}); 4.44 (d, *J* = 7.8 Hz, 0.50H,
14
15 H_{1α}); 4.37 (d, *J* = 6.9, 1H); 3.91 (s, 3H); 3.80-3.34 (m, 13H); 2.50 (m, 2H); 2.12 (m, 2H); 2.06 (s,
16
17 3H); 1.80 (m, 2H); 1.02-0.95 (m, 12H) ; ¹³C NMR (75 MHz, CD₃OD) : δ = 174.2, 171.4, 171.4,
18
19 169.3, 165.4, 155.5, 140.1, 140.0, 139.9, 139.7, 139.5, 133.5, 129.4, 129.3, 129.2, 129.0, 128.9,
20
21 128.7, 128.5, 126.8, 124.3, 121.0, 113.4, 104.8 (C_{1Hα}), 98.1 (C_{1Hβ}), 85.7, 83.0, 81.4, 80.6, 78.9,
22
23 76.3, 76.1, 75.7, 75.5, 71.6, 73.9, 71.6, 70.6, 68.2, 67.0, 59.9, 58.8, 56.9, 38.0, 37.8, 37.4, 32.0,
24
25 31.9, 31.4, 30.3, 23.6, 19.7, 18.9, 18.8, 18.1, 17.9 ; MS (ESI, ion polarity negative, MeOH) : m/z:
26
27 983.53 [M+H]⁺.
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34 Same procedure as described for **12** from *N*-Boc-L-Ala-OH (102.6 mg, 0.55 mmol, 2 eq.) and
35
36 **16a** (300 mg, 0.273 mmol) to afford **18** as a white solid (210 mg, 68%, α/β 50/50). R_f = 0.20
37
38 (CH₂Cl₂/CH₃OH : 95/5) ; ¹H NMR (300 MHz, CD₃OD) : δ = 8.04 (d, *J* = 2.8 Hz, 1H); 7.85 (m,
39
40 1H); 7.40-7.19 (m, 15H); 7.11 (m, 1H); 5.00-4.69 (m, 6.50 H, 6H+H_{1β}); 4.43 (d, *J* = 7.9 Hz,
41
42 0.50H, H_{1α}); 4.38 (d, *J* = 6.9 Hz, 1H); 4.22-4.06 (m, 2H); 3.95 (s, 3H); 3.84-3.28 (m, 12H); 2.67-
43
44 2.44 (m, 2H); 2.25-2.11 (m, 5H); 1.83 (m, 2H); 1.44 (s, 9H); 1.30 (m, 3H); 1.05 (m, 6H); 0.93
45
46 (m, *J* = 6.5 Hz, 6H) ; ¹³C NMR (75 MHz, CDCl₃) : δ = 172.8, 172.7, 171.1, 171.0, 169.0, 165.3,
47
48 158.5, 153.1, 138.8, 138.5, 138.4, 138.1, 133.7, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.4,
49
50 124.9, 122.5, 117.8, 111.8, 103.6 (C_{1Hα}), 97.1 (C_{1Hβ}), 84.5, 82.1, 82.0, 80.1, 80.0, 77.7, 75.5,
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52 75.4, 74.9, 74.7, 74.6, 73.2, 70.2, 70.0, 69.9, 67.9, 67.6, 67.4, 66.7, 58.5, 56.4, 55.9, 50.3, 37.4,
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37.3, 33.0, 31.0, 30.9, 29.2, 28.2, 24.2, 19.3, 18.9, 18.2, 17.7 ; HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+Na]⁺, calcd for C₆₁H₈₃N₇O₁₅Na 1176.5839; found 1176.5827.

tert-Butyl N-[(1*S*)-2-[[[(1*S*)-1-[3-[(3*S*,4*S*,5*R*)-6-[[3-[[[(1*S*)-1-[[5-acetamido-2-methoxybenzoyl]amino]carbamoyl]-5-(benzyloxycarbonylamino)pentyl]amino]-3-oxo-propoxy]methyl]-3,4,5-tribenzyloxy-tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methylpropyl]amino]-1-methyl-2-oxo-ethyl]carbamate (19).

Same procedure as described for **18** from **17** (200 mg, 0.16 mmol) to afford the TFA salt of the free amine **17a** as a white solid (207 mg, quantitative, α/β 50/50). R_f = 0 (CH₂Cl₂/CH₃OH : 95/5) ; ¹H NMR (300 MHz, CD₃OD) : δ = 8.14 (m, 1H) ; 7.74 (d, *J* = 9.0 Hz, 1H); 7.35 – 7.23 (m, 20H); 7.12 (d, *J* = 9.0 Hz, 1H); 5.03 (s, 2H); 4.88 – 4.48 (m, 7.50H, 7H+H_{1 β}); 4.41 (d, *J* = 7.9 Hz, 0.50H, H_{1 α}); 3.90 (s, 3H); 3.89 – 3.20 (m, 13H); 3.07 (m, 2H); 2.55 (m, 2H); 2.13 (m, 1H), 2.08 (m, 3H); 1.93– 1.75 (m, 4H); 1.51 (m, 4H); 1.02 (m, 6H) ; ¹³C NMR (75 MHz, CD₃OD) : δ = 174.2, 172.2, 171.5, 169.4, 165.5, 158.8, 155.6, 140.1, 139.9, 139.7, 138.4, 133.5, 129.4-128.6, 126.8, 124.3, 120.9, 113.5, 104.8 (C_{1H α}), 98.0 (C_{1H β}), 85.7, 83.4, 83.0, 81.6, 79.0, 76.5, 76.1, 75.9, 75.6, 73.9, 71.6, 70.7, 68.5, 68.3, 67.3, 67.0, 59.9, 57.0, 53.4, 41.5, 37.8, 37.5, 32.7, 31.4, 30.4, 24.0, 23.7, 18.9, 18.1, 18.0; HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+H]⁺, calcd for C₆₂H₈₀N₇O₁₄ 1146.5763; found 1146.5752.

Same procedure as described for **12** from *N*-Boc-L-Ala-OH (59.5 mg, 0.32 mmol) and **17a** (200 mg, 0.16 mmol) to afford **19** as a white solid (152 mg, 73%, α/β 50/50). R_f = 0.30 (CH₂Cl₂/CH₃OH : 95/5) ; ¹H NMR (300 MHz, CD₃OD) : δ = 8.05 (m, 1H) ; 7.82 (s, 1H) ; 7.38 – 7.23 (m, 20H); 7.11 (m, 2H); 5.05 (m, 2H); 4.91 – 4.59 (m, 6.50H, 6H+H_{1 β}); 4.49 (m, 1H); 4.40 (d, *J* = 7.8 Hz, 0.50H, H_{1 α}); 4.10 (m, 2H); 3.92 (s, 3H); 3.89 (m, 1H); 3.76 – 3.31 (m, 11H); 3.09 (s, 2H); 2.54 (s, 2H); 2.09 (m, 3H); 2.02 (m, 1H); 1.91 – 1.78 (m, 4H); 1.49 (m, 4H); 1.41 (s,

9H); 1.28 (s, 3H); 0.91 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 175.8, 174.2, 172.2, 172.0, 171.5, 169.4, 165.5, 164.3, 158.7, 155.7, 140.1, 138.6, 133.5, 129.4, 129.3, 129.2, 129.0, 128.9, 128.5, 126.9, 124.3, 120.9, 113.5, 104.8 ($\text{C}_{1\text{H}\alpha}$), 98.0 ($\text{C}_{1\text{H}\beta}$), 83.4, 81.6, 80.8, 79.1, 76.5, 76.1, 75.9, 73.9, 71.6, 70.7, 68.5, 67.3, 67.2, 60.0, 57.0, 53.4, 51.7, 44.6, 41.5, 37.9, 37.5, 32.6, 32.1, 30.6, 30.4, 28.7, 23.9, 23.7, 19.1, 18.4, 18.0, 17.9; MS (ESI, ion polarity positive, MeOH) : m/z: 1339,65 $[\text{M}+\text{Na}]^+$. HRMS (TOF, ESI, ion polarity positive, $\text{H}_2\text{O}/\text{MeOH}$): m/z $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{70}\text{H}_{92}\text{N}_8\text{O}_{17}\text{Na}$ 1339.6478; found 1339.6483. HPLC purity: TR (α,β) = 23.54, 23.87 min., 93.5 %.

tert-Butyl N-[(1*S*)-2-[[[(1*S*)-1-[3-[(3*S*,4*S*,5*S*)-6-[[3-[[[(1*S*)-1-[[[5-acetamido-2-methoxybenzoyl]amino]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]methyl]-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methyl-propyl]amino]-1-methyl-2-oxo-ethyl]carbamate (20). To a stirred solution of **18** (105 mg, 0.091 mmol) in CH_3OH (3 mL) was added Pd/C (25 mg, 25% mass). The reaction flask was purged three times with hydrogen, and stirring was maintained under hydrogen atmosphere at room temperature for 2 days. Upon completion of the reaction monitored by TLC, the solution was filtered through a pad of Celite which was washed several times with CH_3OH . Then, the filtrate was concentrated under reduced pressure to provide **20** (70.7 mg, 88%, α/β 50/50) as a white solid. R_f = 0 ($\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$: 95/5); ^1H NMR (300 MHz, CD_3OD) : δ = 7.97 (s, 1H); 7.77 (dd, J = 9.0, 2.2 Hz, 1H); 7.07 (d, J = 9.0 Hz, 1H); 4.71 (d, J = 3.6 Hz, 0.50H, $\text{H}_{1\beta}$); 4.35 (d, J = 6.9 Hz, 1H); 4.21 (d, J = 7.8 Hz, 0.50H, $\text{H}_{1\alpha}$); 4.08 (m, 1H); 3.93 (s, 3H); 3.75-3.24 (m, 12H); 2.52 (m, 2H); 2.13 (m, 1H); 2.08 (s, 3H); 2.00 (m, 1H); 1.75 (m, H); 1.39 (s, 9H); 1.26 (d, J = 7.2 Hz, 3H); 1.01 (m, 6H); 0.89 (d, J = 6.7 Hz, 6H); ^{13}C NMR (75 MHz, CD_3OD) : δ = 175.6, 174.2, 173.2, 171.7, 171.6, 165.5, 155.6, 155.6, 133.4, 126.9, 124.3, 121.1, 113.4, 104.3 ($\text{C}_{1\text{H}\alpha}$), 100.2 ($\text{C}_{1\text{H}\beta}$), 80.7,

77.9, 76.7, 75.0, 73.4, 72.5, 71.6, 71.5, 71.3, 71.2, 68.7, 68.6, 67.2, 60.1, 58.8, 58.7, 57.0, 51.7, 37.9, 37.4, 32.0, 32.2, 32.0, 30.0, 28.7, 23.6, 19.7, 18.8, 18.7, 17.9 ; HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+Na]⁺, calcd for C₄₀H₆₅N₇O₁₅Na 906.4436; found 906.4421. HPLC purity: TR (α, β) = 17.56 min., 97.1 %.

tert-Butyl N-[(1S)-2-[[[(1S)-1-[3-[(3S,4S,5S)-6-[[3-[[[(1S)-1-[[[(5-acetamido-2-methoxybenzoyl)amino]carbamoyl]-5-amino-pentyl]amino]-3-oxo-propoxy]methyl]-3,4,5-trihydroxy-tetrahydropyran-2-yl]oxypropylcarbamoyl]-2-methyl-propyl]amino]-1-methyl-2-oxo-ethyl]carbamate (21). Same procedure as described for **20** from **19** (111 mg, 0.083 mmol) in CH₃OH (3 mL) except that CH₃CO₂H was added (1 mL) to provide **21** (61.6 mg, 75%, α/β 50/50) as a white solid. R_f = 0 (CH₂Cl₂/ CH₃OH : 95/5); ¹H NMR (300 MHz, CD₃OD) : δ = 8.12 (s, 1H); 7.73 (s, 1H); 7.13 (d, *J* = 8.6 Hz, 1H); 4.76 (d, *J* = 3.6 Hz, 0.50H, H_{1β}); 4.54 (m, 1H), 4.26 (d, *J* = 7.6 Hz, 0.50H, H_{1α}); 4.13 (m, 2H); 3.97 (s, 3H); 3.89 – 3.57 (m, 6H); 3.34 – 3.26 (m, 5H); 3.17 (m, 1H); 2.97 (m, 2H); 2.55 (m, 2H); 2.12 (s, 3H); 2.04 (m, 1H); 1.96 (s, 8H, 2H + CH₃CO₂H); 1.78 (m, 4H); 1.56 (m, 2H); 1.43 (s, 9H); 1.29 (m, 3H); 0.93 (d, *J* = 6.6 Hz, 6H) ; ¹³C NMR (75 MHz, CD₃OD) : δ = 177.3, 175.8, 174.4, 173.3, 171.6, 157.8, 155.7, 133.5, 130.5, 126.9, 124.4, 121.0, 113.5, 104.4 (C_{1Hα}), 100.3 (C_{1Hβ}), 8.7, 77.9, 76.7, 75.1, 73.5, 72.6, 71.6, 71.5, 71.1, 68.6, 67.2, 60.1, 57.0, 53.0, 51.7, 45.8, 40.5, 37.9, 37.7, 37.4, 32.5, 32.2, 30.2, 28.7, 28.1, 26.9, 23.7, 22.1, 19.7, 19.3, 18.6, 17.9; HRMS (TOF, ESI, ion polarity positive, H₂O/ MeOH): m/z [M+H]⁺, calcd for C₄₁H₆₉N₈O₁₅ 913.4882; found 913.4883, m/z [M+Na]⁺, calcd for C₄₁H₆₈N₈O₁₅Na 935.4702, found 935.4708 ; HPLC purity: TR (α, β) = 12.8, 13.1 min., 88.9 %.

(2S)-N-[3-[(3S,4S,5S)-6-[[3-[[[(1S)-1-[[[(5-Acetamido-2-methoxybenzoyl)amino]carbamoyl]-2-methyl-propyl]amino]-3-oxo-propoxy]methyl]-3,4,5-tribenzyloxy-tetrahydropyran-2-yl]oxypropyl]-2-[[[(2S)-2-aminopropanoyl]amino]-3-

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2
3 **methyl-butanamide (22)**. Same procedure as described for **16a** from **18** (38 mg, 0.033 mmol) in
4
5 dry CH₂Cl₂ (270 μL) to yield **22** as a white solid (40 mg, quantitative, α/β 50/50). R_f = 0
6
7 (CH₂Cl₂/ CH₃OH: 95/5); ¹H NMR (300 MHz, CD₃OD) : δ = 8.12 (m, 1H); 7.77 (d, *J* = 8.9 Hz,
8
9 1H); 7.35-7.26 (m, 15H); 7.10 (d, *J* = 8.9 Hz, 1H); 5.07-4.61 (m, 6.50H, 6H+H_{1β}); 4.44 (d, *J* =
10
11 7.9 Hz, 0.50H, H_{1α}); 4.40 (m, 1H); 4.17 (m, 1H); 4.02 (m, 1H); 3.94 (s, 3H); 3.86-3.35 (m, 12H);
12
13 2.56 (m, 2H); 2.34-1.98 (m, 5H); 1.84 (m, 2H); 1.51-1.47 (m, 3H); 1.30 (s, 3H); 0.99 (m, 12H) ;
14
15 ¹³C NMR (75 MHz, CD₃OD) : δ = 174.1, 173.0, 171.0, 171.5, 171.0 165.4, 155.6, 140.2, 140.0,
16
17 139.9, 139.7, 139.5, 133.5, 129.4-128.5, 126.8, 124.3 , 121.0, 113.4, 104.8 (C_{1Hα}), 98.0 (C_{1Hβ}),
18
19 85.7, 83.2, 83.0, 81.4, 79.0, 76.4, 76.0, 75.9, 75.6, 73.9, 71.7 70.6, 68.5, 68.3, 68.2, 67.3, 60.7,
20
21 58.8, 56.9, 50.3, 49.9, 38.0, 37.6, 37.5, 32.0, 31.9, 30.4, 30.4, 23.6, 19.8, 19.0, 18.9, 18.7, 17.8 ;
22
23 HRMS (TOF, ESI, ion polarity positive, H₂O/MeOH): *m/z* [M+H]⁺, calcd for C₅₆H₇₆N₇O₁₃
24
25 1054.5501; found 1054.5524, *m/z* [M+Na]⁺, calcd for C₅₆H₇₅N₇O₁₃Na 1076.5321; found
26
27 1076.5360; HPLC purity: TR (α,β) = 18.77, 19.24 min., 96.1 %.

28
29 **Benzyl N-[(5S)-6-[2-(5-acetamido-2-methoxy-benzoyl)hydrazino]-5-[3-[(3R,4S,5S)-6-[3-**
30
31 **[(2S)-2-[(2S)-2-aminopropanoyl]amino]-3-methyl-butanoyl]amino]propoxy]-3,4,5-**
32
33 **tribenzyloxy-tetrahydropyran-2-yl]methoxy]propanoylamino]-6-oxo-hexyl]carbamate (23)**.

34
35 Same procedure as described for **16a** from **19** (38 mg, 0.029 mmol) in dry CH₂Cl₂ (220 μL), to
36
37 afford **23** as a white solid (35.5 mg, quantitative, α/β 50/50). R_f = 0 (CH₂Cl₂/ CH₃OH : 95/5); ¹H
38
39 NMR (300 MHz, CDCl₃) : δ = 11.10 (sl, 1H) ; 10.65 (m, 1H); 9.52 (m, 1H); 8.50-8.01 (m, 7H);
40
41 7.32- 7.25 (m, 21H,); 6.84 (m, 1H); 5.25-4.49 (m, 11.50H, 11H+H_{1β}); 4.36 (m, 1.50H, 1H+H_{1α});
42
43 3.87 (s, 3H); 3.78 – 2.93 (m, 16H); 2.51 (s, 2H); 2.11 (s, 3H); 1.96- 1.81 (m, 5H); 1.50 (s, 3H);
44
45 1.44 (m, 4H); 0.88-0.85 (m, 6H) ; ¹³C NMR (75 MHz, CDCl₃) : δ = 173.2, 172.0, 171.5, 171.3,
46
47 169.9, 165.5, 158.8, 153.6, 138.6, 138.2, 138.6, 138.0, 136.6, 136.5, 135.9, 133.0, 128.4,
48
49
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51
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3 128.3,127.9, 127.8, 127.7, 127.6, 125.8, 122.9, 117.6, 111.9, 103.9 (C_{1H α}), 97.1 (C_{1H β}), 84.4,
4
5 82.1, 81.8, 80.0, 78.2, 77.5, 75.5, 74.9, 74.7, 74.6, 72.9, 69.6, 67.6, 66.5, 59.3, 59.1, 56.3 , 51.3,
6
7 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,
8
9 ESI, ion polarity positive, H₂O/ MeOH): m/z [M+H]⁺, calcd for C₆₅H₈₅N₈O₁₅ 1217.6134; found
10
11 1217.6168, m/z [M+Na]⁺, calcd for C₆₅H₈₄N₈O₁₅Na 1239.5954; found 1239.5972, HPLC purity:
12
13 TR (α,β) = 19.24, 19.67 min., 91.1 %.

17 **Fluorescence-detected Thioflavin-T binding assay**

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19 Thioflavin T was obtained from Sigma. A β ₁₋₄₂ was purchased from American Peptide. The
20
21 peptide was dissolved in an aqueous 1% ammonia solution to a concentration of 1 mM and then,
22
23 just prior to use, was diluted to 0.2 mM with 10 mM Tris-HCl, 100 mM NaCl buffer (pH 7.4).
24
25 Stock solutions of glycopeptides were dissolved in DMSO with the final concentration kept
26
27 constant at 0.5% (v/v).
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31 Thioflavin T fluorescence was measured to evaluate the development of A β ₁₋₄₂ fibrils over
32
33 time using a fluorescence plate reader (Fluostar Optima, BMG labtech) with standard 96-well
34
35 black microtiter plates. Experiments were started by adding the peptide (final A β ₁₋₄₂
36
37 concentration equal to 10 μ M) into a mixture containing 40 μ M Thioflavin T in 10 mM Tris-
38
39 HCl, 100 mM NaCl buffer (pH 7.4) with and without the tested compounds at different
40
41 concentrations (100, 50, 10, 1 μ M) at room temperature. The Th-T fluorescence intensity of each
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43 sample (performed in duplicate or triplicate) was recorded with 440/485 nm excitation/emission
44
45 filters set for 42 hours performing a double orbital shaking of 10 s. before the first cycle. The
46
47 fluorescence assays were performed between 2 and 4 times on different days, with the same
48
49 batch of peptide. The ability of compounds to inhibit/accelerate A β ₁₋₄₂ aggregation was
50
51 assessed considering both the time of the half-life of aggregation (t_{1/2}) and the intensity of the
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3 experimental fluorescence plateau (F). The relative extension (or reduction) of t1/2 is defined as
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5 the experimental t1/2 in the presence of the tested compound relative to the one obtained without
6
7 the compound and is evaluated as the following percentage: $t_{1/2}(A\beta + \text{compound}) / t_{1/2}(A\beta) \times$
8
9 100. The decrease (or increase) of the experimental plateau is defined as the intensity of
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11 experimental fluorescence plateau observed with the tested compound relative to the value
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13 obtained without the compound and is evaluated as the absolute value of following percentage :
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15 $|FA\beta - FA\beta + \text{compound}| / FA\beta \times 100$ (a decrease is indicated with a (-) and an increase with a
16
17 (+).

21 22 **Transmission electron microscopy**

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24 Samples were prepared under the same conditions as in the ThT-fluorescence assay. Aliquots
25
26 of A β_{1-42} (10 μ M in 10 mM Tris-HCl, 100 mM NaCl NaCl, pH 7.4 in the presence and absence
27
28 of the tested compounds) were adsorbed onto 300-mesh carbon grids for 2 min, washed and
29
30 dried. The samples were negatively stained for 45 s. on 2 % uranyl acetate in water. After
31
32 draining off the excess of staining solution and drying, images were obtained using a ZEISS 912
33
34 Omega electron microscope operating at an accelerating voltage of 80 kV.
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36

37 38 **Capillary electrophoresis**

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40 Sample preparation: the commercial A β_{1-42} was dissolved upon reception in 0.16% NH₄OH
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42 (at 2 mg/mL) for 10 minutes at 20°C, followed by an immediate lyophilization. The dried sample
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44 was then stored at -20°C until use.
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48 CE experiments were carried out with a P/ACE TM MDQ Capillary Electrophoresis System
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50 (Beckman Coulter Inc., Brea, CA, USA) equipped with a photodiode array detector. UV
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52 Detection was performed at 190 nm. The sample (as previously described) was reconstituted by
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54 dissolution in 20 mM phosphate buffer pH 7.4 containing DMSO (control or stock solutions of
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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 μ M regardless the peptide/compound ratio.

For the CE separation of A β oligomers, fused silica capillary 60 cm (10.2 cm to the detector) 50 μ m I.D. were 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 NaOH 1 M, 3.5 min with water, 1 min with DMSO 10 %, 1 min with SDS 50 mM, and equilibrated with running buffer for 3 min.

NMR spectroscopy

NMR experiments were recorded on a Bruker Avance III 500 MHz spectrometer equipped with a $^1\text{H}/^{13}\text{C}/^{15}\text{N}$ TCI cryoprobe with Z-axis gradient. NMR spectra were processed and analysed with TopSpin software (Bruker).

The conformation of **3 β** 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 ^{13}C 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 - ^{13}C HSQC, and 2D ^1H - ^{13}C HMBC spectra. ^1H and ^{13}C chemical shifts were calibrated using DSS (sodium 4,4-dimethyl-4-silapentane-1-sulfonate) as an internal reference. The ^1H and ^{13}C resonance assignments of **3 β** are listed in Tables S1 and S2. Vicinal 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 °C and 30 °C.

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3 Samples of A β ₁₋₄₂ in the absence or in the presence of **3 β** were prepared in Shigemi tubes (280
4 μ L volume) in 50 mM sodium phosphate, pH 7.4 containing 10% D₂O. Synthetic A β ₁₋₄₂ peptide
5 was used in NMR experiments, with the exception of 2D HSQC experiments requiring ¹⁵N, ¹³C-
6 labelled recombinant A β ₁₋₄₂. Solid phase peptide synthesis of A β ₁₋₄₂ was performed at the
7 Institut de Biologie Intégrative (IFR83- Université Pierre et Marie Curie). Recombinant A β ₁₋₄₂
8 was obtained according to the protocol of Walsh et al.³⁵ NMR experiments were acquired at
9 5 °C. 2D NOESY experiments were recorded with a mixing time of 0.2 s. 1D ¹H STD
10 experiments were acquired using a cascade of Gaussian shaped pulses (50 ms pulse, B1 field of
11 0.1 kHz, total duration of 3 s) applied on resonance (−0.7 ppm) and off resonance (+30 ppm),
12 alternatively. The number of scans was set to 320, corresponding to an experiment duration of 50
13 min. 1D ¹H WaterLOGSY (water-ligand observed via gradient spectroscopy) experiments were
14 recorded using a Gaussian pulse of 20 ms duration for selective inversion of water magnetization
15 and a mixing period of 0.5 s. The recycling delay was set to 2 s and the total number of scans
16 was 1200, corresponding to an experimental time of 1 hour.

36 **SPR experiments**

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38 For these studies we used the Biacore T100 (GE Healthcare, France) apparatus, which has 4
39 parallel flow channels. A β ₁₋₄₂ peptide was immobilized on the carboxy-terminated dextran matrix
40 on gold surface sensor chip (CM5 sensor chip, GE Healthcare, France) by an optimized amine
41 coupling method. Briefly, the surface was treated with a mixture of 0.4 M EDC and 0.1 M NHS
42 (1/1) in water for 7 min. at 10 μ L/min. Then, a freshly prepared A β ₁₋₄₂ solution (0.05 μ M) in a 10
43 mM sodium acetate buffer (pH 4.6) was injected 4 times during 15 min. each at 10 μ L/min on
44 the NHS-activated surface. Then, a final injection of ethanolamine was done to block the non-
45 linked activated amine. A reference surface was prepared using the same immobilization
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3 procedure but with an ethanolamine injection instead of the peptide (blank surface). At that point
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5 of the process, the fixation lead to a SPR signal of 3000 RU. A rinsing step using an aqueous
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7 solution of $\text{NH}_4\text{OH}\cdot\text{H}_2\text{O}$ 0.1 % (9 injections of 1 min. each at 30 $\mu\text{L}/\text{min.}$) was performed in
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9 order to remove from the surface all the $\text{A}\beta_{1-42}$ aggregates that may have been formed during the
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11 immobilization step. After these rinsing steps, the chip gave a signal of about 1500 RU.
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15 Binding of compounds **3**, **1** and curcumin to $\text{A}\beta_{1-42}$ fixed on the SPR chips : Solutions of
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17 compounds in 150 mM PBS containing 2% DMSO solubilized in DMSO were injected on the
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19 $\text{A}\beta_{1-42}$ surface for 1 min. at 80 $\mu\text{L}/\text{min.}$ The rinsing step was performed using the running buffer
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21 for 5 min. and the regeneration step using the aqueous solution of $\text{NH}_4\text{OH}\cdot\text{H}_2\text{O}$ 0.1 % (3 times
22
23 for 5 min at 30 $\mu\text{L}/\text{min.}$). The range of concentrations started from 12.5 μM and ended at 200
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25 μM in PBS (150 mM), 2% DMSO. A DMSO solvent correction was applied to the raw signals
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27 and non-specific signal was subtracted using the blank channel.
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31 32 Cell toxicity

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34 SH-SY5Y neuroblastoma cells were grown in low serum Optimem (Life Technologies) for 24
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36 hours at 37°C, 5% CO_2 in a 96 well plate at 20 000 cells per well. $\text{A}\beta_{1-42}$ was dissolved in sterile
37
38 PBS at 50 μM concentration in the presence of 1, 5, 10 and 50 μM of either **1** or **3** for 24 hours at
39
40 room temperature, along with a control incubation with no inhibitor. After the 24 hour period,
41
42 media was removed from the cells and replaced with Optimem containing the pre-incubated $\text{A}\beta_{1-}$
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44 $_{42}$ plus inhibitor diluted one in ten (5 μM $\text{A}\beta$ final concentration) in quadruplicate. The cells
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46 were incubated for a further 24 hours as before and the cell viability (MTS assay) and cell
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48 proliferation (LDH assay) assessed using the CellTiter 96® Aqueous One Solution Cell
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50 Proliferation Assay (Promega) and CytoTox 96® Non-Radioactive Cytotoxicity Assay
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52 (Promega) respectively. The assays were repeated twice and representative samples are shown.
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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 microlitre 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 hour incubation at 37°C 100 μL samples were run on the same gradient and monitored as before.

ASSOCIATED CONTENT

Supporting Information. NMR assignments of **3 β** ; representative NMR spectra and HPLC purities, experimental procedure for fluorescence-detected ThT binding assay on A β_{1-42} and IAPP; representative curves of ThT fluorescence assays; experimental procedure for TEM, CE, NMR and SPR.

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ABBREVIATIONS

A β , 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 relationships; DMTMM, [4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate]; DMAP, 4-dimethylaminopyridine.

REFERENCES

- (1) (a) Alzheimer's disease international. World Alzheimer Reports. <http://www.alz.co.uk/research/world-report> (accessed december 14th, 2015). (b) Mucke, L. Neuroscience : Alzheimer's disease. *Nature* **2009**, *461*, 895-897.
- (2) (a) Goedert, M.; Spillantini, M. G. A Century of Alzheimer's Disease. *Science* **2006**, *314*, 777-780. (b) Haas, C. ; Selkoe, D. J. Soluble protein oligomers in neurodegeneration : lessons from the Alzheimer amyloid β -peptide. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 101-112.
- (3) Cohen, S. I. A.; Linse, S.; Luheshi, M.; Hellstrand, E.; White, D. A.; Rajah, L.; Otzen, D. L.; Vendruscolo, M.; Dobson, C. M.; Knowles, T. P. J. Proliferation of amyloid- β 42 aggregates occurs through a secondary nucleation mechanism. *Proc. Natl. Acad. Sci. USA.* **2013**, *110*, 9758-9763.
- (4) Matsumura, S.; Shinoda, K.; Yamada, M.; Yokojima, S.; Inoue, M.; Ohnishi, T; Shimada, T.; Kikuchi, K.; Masui, D.; Hashimoto, S.; Sato, M.; Ito, A.; Akioka, M.; Takagi, S.; Nakamura, Y.; Nemoto, K.; Hasegawa, Y.; Takamoto, H.; Inoue, H.; Nakamura, S.; Nabeshima, Y.; Teplow, D.B.; Kinjo, M.; Hoshi, M. Two distinct amyloid β -protein ($A\beta$) assembly pathways leading to oligomers and fibrils identified by combined fluorescence correlation spectroscopy, morphology, and toxicity analyses. *J. Biol. Chem.* **2011**, *286*, 11555-11562.
- (5) Jeong, J. S.; Ansaloni, A.; Mezzenga, R.; Lashuel, H. A.; Dietler, G. Novel mechanistic insight into the molecular basis of amyloid polymorphism and secondary nucleation during amyloid formation. *J. Mol. Biol.* **2013**, *425*, 1765-1781.

1
2
3 (6) Lührs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Döbeli, H.; Schubert, D.;
4 Riek, R. 3D structure of Alzheimer's amyloid- β (1-42) fibrils. *Proc. Natl. Acad. Sci. USA.* **2005**,
5
6 *102*, 17342-17347.
7
8

9
10
11 (7) Laganowsky, A.; Liu, C.; Sawaya, R. M.; Whitelegge, J. P.; Park, J.; Zhao, M. Atomic
12 view of a toxic amyloid small oligomer. *Science* **2012**, *335*, 1228-1231.
13
14

15
16
17 (8) Yu, L.; Edalji, R.; Harlan, J. E.; Holzman, T. F.; Pereda Lopez, A.; Labkovsky, B.; Hillen,
18 H.; Barghorn, S.; Ebert, U.; Richardson, P.L.; Miesbauer, L.; Solomon, L.; Bartley, D.; Walter,
19 K.; Johnson, R.W.; Hajduk, P.J. and Olejniczak, E.T. Structural characterization of a soluble
20 amyloid β -peptide oligomer. *Biochemistry* **2009**, *48*, 1870-1877.
21
22
23

24
25
26
27 (9) Ahmed, M.; Davis, J.; Aucoin, D.; Sato, D.; Ahuja, S.; Aimoto, S.; Elliott, J. J.; Van
28 Nostrand W. E.; O Smith. Structural conversion of neurotoxic amyloid- β 1-42 oligomers to
29 fibrils. *Nat. Struct. Mol. Biol.* **2010**, *17*, 561-567.
30
31
32

33
34
35 (10) Wälti M. A.; Orts J.; Vçgeli B.; Campioni S.; Riek R. Solution NMR Studies of
36 recombinant A β (1-42): from the presence of a micellar entity to residual β -sheet structure in the
37 soluble species. *ChemBioChem* **2015**, *16*, 659-669.
38
39
40

41
42
43 (11) Dahlgren, K. N.; Manelli, A. M.; Blaine Stine, W.; Baker, L. K.; Krafft, G. A; LaDu, M. J.
44 Oligomeric and fibrillar species of amyloid- β peptides differentially affect neuronal viability. *J.*
45 *Biol. Chem.* **2002**, *277*, 32046-32053.
46
47
48

49
50
51 (12) Jan, A.; Gokce, O.; Luthi-Carter R.; Lashuel, H. A. The ratio of Monomeric to
52 Aggregated forms of A β 40 and A β 42 is an important determinant of amyloid- β aggregation,
53 fibrillogenesis, and toxicity. *J. Biol. Chem.* **2008**, *283*, 28176-28189.
54
55
56
57
58
59
60

1
2
3 (13) Ono, K.; Condrón, M. M.; Teplow, D. B. Structure-neurotoxicity relationships of amyloid
4 β -protein oligomers. *Proc. Natl. Acad. Sci. USA*. **2009**, *106*, 14745-14750.
5
6

7
8
9 (14) Shankar, G. M.; Li, S.; Mehta, T. H.; Garcia-Munoz A.; Shepardson, N. E.; Smith I.;
10 Brett, F.M.; Farrell, M.A.; Rowan, M.J.; Lemere, C.A.; Regan, C.M.; Walsh, D.M.; Sabatini,
11 B.L. and Selkoe, D.J. Amyloid β -protein dimers isolated directly from Alzheimer brains impair
12 synaptic plasticity and memory. *Nat. Med.* **2008**, *14*, 837-842.
13
14
15

16
17
18 (15) Prangkio, P.; Yusko, E. C.; Sept, D.; Yang, J.; Mayer, M. Multivariate analyses of
19 amyloid-beta oligomer populations indicate a connection between pore formation and
20 cytotoxicity. *PLoS ONE* **2012**, *7*, 47261.
21
22
23

24
25
26 (16) Cizas, P.; Budvytyte, R.; Morkuniene, R.; Moldovan, R.; Broccio, M.; Lösche, M.;
27 Niaura, G.; Valincius, G.; Borutaite, V. Size-dependent neurotoxicity of β -amyloid oligomers.
28
29
30
31
32 *Arch. Biochem. Biophys.* **2010**, *496*, 84-92.
33

34
35 (17) Mayes, J.; Tinker-Mill, C.; Kolosov, O.; Zhang, H.; Tabner, B. J.; Allsop, D. β -Amyloid
36 fibrils in Alzheimer's disease are not inert when bound to copper ions but can degrade hydrogen
37 peroxide and generate reactive oxygen species. *J Biol Chem.* **2014**, *289*, 12052-12062.
38
39
40

41
42
43 (18) Cohen, S. I. A.; Linse, S.; Luheshi, L. M.; Hellstrand, E.; White, D. A.; Rajah, L.;
44 Otzen, D. E.; Vendruscolo, M.; Dobson, C. M.; Knowles, T. P. J. Proliferation of amyloid- β 42
45 aggregates occurs through a secondary nucleation mechanism. *Proc Natl Acad Sci U S A.* **2013**,
46
47
48
49
50
51
52 *110*, 9758-9763.

53 (19) (a) Belluti, F.; Rampa, A.; Gobbi, S.; Bisi, A. Small-molecule inhibitors/modulators of
54 amyloid- β peptide aggregation and toxicity for the treatment of Alzheimer's disease: a patent
55
56
57
58
59
60

1
2
3 review (2010 - 2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 581-596. (b) Härd, T.; Lendel, C.
4
5 Inhibition of amyloid formation. *J. Mol. Biol.* **2012**, *421*, 441-465. (c) Doig, A. J.; Derreumaux
6
7 P. Inhibition of protein aggregation and amyloid formation by small molecules. *Curr. Opin.*
8
9 *Struct. Biol.* **2015**, *30*, 50-56.
10
11

12
13 (20) (a) Stains, C.I.; Mondal, K.; Ghosh, I. Molecules that target beta-Amyloid.
14
15 *ChemMedChem.* **2007**, *2*, 1674-1692. (b) Takahashi, T.; Mihara, H. Mimetics. Peptide and
16
17 protein mimetics inhibiting amyloid peptide aggregation. *Acc. Chem. Res.* **2008**, *41*, 1309-1318.
18
19 (c) Neddenriep, B.; Calciano, A.; Conti, D.; Sauve, E.; Paterson, M.; Bruno, E.; Moffet, D. A.
20
21 Short peptides as inhibitors of amyloid aggregation. *Open Biotechnol. J.* **2011**, *5*, 39-46. (d) Luo,
22
23 J.; Abrahams, J. P. Cyclic peptides as inhibitors of amyloid fibrillation. *Chem. Eur. J.* **2014**, *20*,
24
25 2410-2419.
26
27
28
29

30
31 (21) Cheng, P.-N.; Liu, C.; Zhao, M.; Eisenberg, D.; Nowick, J. S. Amyloid β -sheet mimics
32
33 that antagonize protein aggregation and reduce amyloid toxicity. *Nat. Chem.* **2012**, *4*, 927-933.
34
35

36
37 (22) Taylor, M.; Moore, S.; Mayes, J.; Parkin, E.; Beeg, M.; Canovi, M.; Gobbi, M.; Mann, D.
38
39 M. A.; Allsop, D. Development of a proteolytically stable retro-inverso peptide inhibitor of β -
40
41 amyloid oligomerization as a potential novel treatment for Alzheimer's disease. *Biochemistry*
42
43 **2010**, *49*, 3261-3272.
44
45

46
47 (23) Arai, T.; Araya, T.; Sasaki, D.; Taniguchi, A.; Sato, T.; Sohma, Y.; Kanai, M. Rational
48
49 Design and identification of a non-peptidic aggregation inhibitor of amyloid- β based on a
50
51 pharmacophore motif obtained from cyclo[-Lys-Leu-Val-Phe-Phe-]. *Angew. Chem. Int. Ed.*
52
53 **2014**, *53*, 8236-8239.
54
55
56
57
58
59
60

1
2
3 (24) (a) Dorgeret, B.; Khémtemourian, L.; Correia, I.; Soulier, J-L; Lequin, O. ; Ongeri, S.
4
5 Sugar-based peptidomimetics inhibit amyloid β -peptide aggregation. *Eur. J. Med. Chem.* **2011**,
6
7 46, 5959-5969. (b) Kaffy, J.; Brinet, D.; Soulier, J-L; Khemtémourian, L.; Lequin, O.; Taverna,
8
9 M.; Crousse, B.; Ongeri, S. Structure-activity relationships of sugar-based peptidomimetics as
10
11 modulators of amyloid β -peptide early oligomerization and fibrillization. *Eur. J. Med. Chem.*
12
13 **2014**, 86, 752-758.
14
15

16
17
18 (25) (a) Gruner, S. A. W.; Truffault, V.; Voll, G.; Locardi, E.; Stöckle, M.; Kessler, H. Design,
19
20 Synthesis, and NMR structure of linear and cyclic oligomers containing novel furanoid sugar
21
22 amino acids. *Chem. Eur. J.* **2002**, 8, 4366-4376. (b) Schweizer, F. Glycosamino acids: building
23
24 blocks for combinatorial synthesis—implications for drug discovery. *Angew. Chem. Int. Ed.* **2002**,
25
26 41, 230-253. (c) Risseueuw, M. D. P.; Overhand, M.; Fleet, G. W. J.; Simone, M. I. A
27
28 compendium of sugar amino acids (SAA): scaffolds, peptide- and glyco-mimetics. *Tetrahedron:*
29
30 *Asymmetry* **2007**, 18, 2001-2010.
31
32
33

34
35
36 (26) Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. An unnatural
37
38 amino acid that mimics a tripeptide β -strand and forms β -sheet like hydrogen-bonded dimers. *J.*
39
40 *Am. Chem. Soc.* **2000**, 122, 7654-7661.
41
42

43
44 (27) (a) Bannwarth, L.; Kessler, A.; Pèthe, S.; Collinet, B.; Merabet, N.; Boggetto, N.; Sicsic,
45
46 S.; Reboud-Ravaux, M.; Ongeri, S. Molecular tongs containing amino acid mimetic fragments :
47
48 new inhibitors of wild-type and mutated HIV-1 protease dimerization. *J. Med. Chem.* **2006**, 49,
49
50 4657-4664. (b) Vidu, A.; Dufau, L.; Bannwarth, L.; Soulier, J-L; Sicsic, S.; Piarulli, U.; Reboud-
51
52 Ravaux, M.; Ongeri, S. Towards the first non peptidic molecular tong inhibitor of wild-type and
53
54 mutated HIV-1 protease dimerization. *ChemMedChem* **2010**, 5, 1899-1906.
55
56
57
58
59
60

1
2
3 (28) (a) Yamanoi, T.; Inoue, R.; Matsuda, S.; Iwao, K.; Oda, Y.; Yoshida, A.; Hamasaki, K.
4 Formation of O-glycosidic linkages from 1-hydroxy sugars by bismuth(III) triflate-catalyzed
5
6
7
8
9
10
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52
53
54
55
56
57
58
59
60

Formation of O-glycosidic linkages from 1-hydroxy sugars by bismuth(III) triflate-catalyzed
dehydrative glycosidation. *Heterocycles* **2009**, *77*, 445-460. (b) Yamazaki, T.; Sugawara, F.;
Ohta, K.; Masaki, K.; Nakayama, K.; Sakaguchi, K.; Sato, N.; Sahara, H.; Fujita, T. Novel
sulfoquinovosylacylglycerol derivative, and use thereof as medicaments. *US. Patent Appl. Publ.*
2002, US 20020052327 A1.

(29) (a) Kaminski, Z.J.; Kolesinska B.; Kolesinska, J.; Sabatino, G.; Chelli, M.; Rovero, P.;
Błaszczuk, M.; Głowka, M.L.; and Papini, A.M. N-Triazinylammonium tetrafluoroborates. A
new generation of efficient coupling reagents useful for peptide synthesis. *J. Am. Chem. Soc.*
2005, *127*, 16912-16920. (b) Jastrzabek K.G.; Subiros-Funosas, R.; Albericio, F.; Kolesinska, B.;
and Kaminski, Z.J. 4-(4,6-Di[2,2,2-trifluoroethoxy]-1,3,5-triazin-2-yl)-4-methylmorpholinium
tetrafluoroborate. Triazine-based coupling reagents designed for coupling sterically hindered
substrates. *J. Org. Chem.* **2011**, *76*, 4506-4513.

(30) Komarova, B. S.; Maryasina, S. S.; Tsvetkov, Y. E.; Nifantiev, N. E. Water-dependent
reduction of carbohydrate azides by dithiothreitol. *Synthesis* **2013**, *45*, 471-478.

(31) LeVine 3rd, H. Quantification of β -sheet amyloid fibril structures with thioflavin T.
Methods Enzymol. **1999**, *309*, 274-284.

(32) Brinet, D.; Kaffy, J.; Oukacine, F.; Glumm, S.; Ongeri, S.; Taverna, M. An improved CE
method for the in vitro monitoring of the challenging early steps of the $A\beta_{1-42}$ peptide
oligomerization: application to anti-Alzheimer's drug discovery. *Electrophoresis* **2014**, *35*, 3302-
3309.

1
2
3 (33) Fawzi, N.L.; Ying, J.; Ghirlando, R.; Torchia, D.A.; Clore, G.M. Atomic-resolution
4 dynamics on the surface of amyloid- β protofibrils probed by solution NMR. *Nature* **2011**, *480*,
5 268-272.
6
7

8
9
10
11 (34) Walsh, D.M.; Thulin, E.; Minogue, A.M.; Gustavsson, N.; Pang, E.; Teplow, D.B.; Linse,
12 S. A facile method for expression and purification of the Alzheimer's disease-associated amyloid
13 β -peptide. *FEBS J.* **2009**, *276*, 1266-1281.
14
15

16
17
18
19 (35) Airoidi, C.; Cardona, F.; Sironi, E.; Colombo, L.; Salmona, M.; Silva, A.; Nicotra, F.; La
20 Ferla, B. cis-Glyco-fused benzopyran compounds as new amyloid- β peptide ligands. *Chem.*
21 *Commun.* **2011**, *47*, 10266-10268.
22
23
24

25
26
27 (36) Taniguchi, A.; Sohma, Y.; Hirayama, Y.; Mukai, H.; Kimura, T.; Hayashi, Y.; Matsuzaki,
28 K.; Kiso, Y. "Click peptide": pH-triggered in situ production and aggregation of monomer
29 Abeta1-42. *ChemBioChem* **2009**, *10*, 710-715.
30
31
32

33
34
35 (37) Canovi, M.; Lucchetti, J.; Stravalaci, M.; Re, F.; Moscatelli, D.; Bigini, P.; Salmona, M.;
36 Gobbi, M. Applications of surface plasmon resonance (SPR) for the characterization of
37 nanoparticles developed for biomedical purposes. *Sensors* **2012**, *12*, 16420-16432.
38
39
40

41
42
43 (38) (a) Amijee, H.; Bate, C.; Williams, A.; Virdee, J.; Jeggo, R.; Spanswick, D.; Scopes,
44 D.I.C.; Treherne, J.M.; Mazzitelli, S.; Chawner, R.; Evers, C.E.; Doig, A.J. The N-methylated
45 peptide SEN304 powerfully inhibits A β (1-42) toxicity by perturbing oligomer formation.
46 *Biochemistry* **2012**, *51*, 8338-8352. (b) Maezawa, I.; Hong, H-S.; Liu, R.; Wu, C-Y.; Cheng, R-
47 H.; Kung, M-P.; Kung, H.F.; Lam, K.S.; Oddo, S.; LaFerla, F.M.; Jin, L-W. Congo red and
48 thioflavin-T analogs detect A β oligomers. *J. Neurochem.* **2008**, *104*, 457-468.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 (39) Stravalaci, M.; Bastone, A.; Beeg, M.; Cagnotto, A.; Colombo, L.; Di Fede, G.;
4 Tagliavini, F.; Cantù, L.; Del Favero, E.; Mazzanti, M.; Chiesa, R.; Salmona, M.; Diomedede, L.;
5 Gobbi, M. Specific recognition of biologically active amyloid- β oligomers by a new surface
6 plasmon resonance-based immunoassay and an in vivo assay in *Caenorhabditis elegans*. *J. Biol.*
7 *Chem.* **2012**, *287*, 27796-27805.

14
15
16 (40) Kai, T.; Zhang, L.; Wang, X.; Jing, A.; Zhao, B.; Yu, X.; Zheng, J.; Zhou, F. Tabersonine
17 inhibits amyloid fibril formation and cytotoxicity of A β (1-42). *ACS Chem. Neurosci.* **2015**, *6*,
18 879-888.

22
23
24 (41) Yang, F.; Lim, G-P.; Begum, A-N.; Ubeda, O-J.; Simmons, M-R.; Ambegaokar, S-S.;
25 Chen, P-P.; Kaye, R.; Glabe, C-G.; Frautschy, S-A.; Cole, G-M. Curcumin inhibits formation of
26 amyloid β oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. *J. Biol. Chem.*
27 **2005**, *280*, 5892-5901.

30
31
32 (42) (a) Smith, M.D.; Cruz, L. Changes to the structure and dynamics in mutations of A β 21-30
33 caused by ions in solution. *J. Phys. Chem. B* **2013**, *117*, 14907-14915. (b) Hochdörffer, K.;
34 März-Berberich, J.; Nagel-Steger, L.; Epple, M.; Meyer-Zaika, W.; Horn, A.H.C.; Sticht, H.;
35 Sinha, S.; Bitan, G.; Schrader, T. Rational design of β -sheet ligands against A β 42-induced
36 toxicity. *J. Am. Chem. Soc.* **2011**, *133*, 4348-4358.

39
40
41 (43) Feng, Y.; Wang, X.-P.; Yang, S.-G.; Wan, Y.-J.; Xi Zhang, Du, X.-T.; Sun, X.-X.; Zhao,
42 M.; Huang, L.; Liu, R.-T. Resveratrol inhibits beta-amyloid oligomeric cytotoxicity but does not
43 prevent oligomer formation. *Neurotoxicology* **2009**, *30*, 986-995.

1
2
3 (44) Sinha, S.; Du, Z.; Maiti, P.; Klärner, F.-G.; Schrader, T.; Wang, C.; Bitan, G. Comparison
4 of Three Amyloid Assembly Inhibitors: The sugar *scyllo*-inositol, the polyphenol
5 epigallocatechin gallate, and the molecular tweezer CLR01. *ACS Chem. Neurosci.* **2012**, *3*, 451-
6 458.
7
8
9
10

11
12
13 (45) Hyunga, S.-J.; DeToma, A. S.; Brender, J. R.; Leec, S.; Vivekanandana, S.; Kochia, A.;
14 Choic, J.-S.; Ramamoorthya, A.; Ruotolo, B. T.; Lima, M. H. Insights into anti-amyloidogenic
15 properties of the green tea extract (-)-epigallocatechin-3-gallate toward metal-associated
16 amyloid- β species. *Proc. Natl. Acad. Sci. USA.* **2013**, *110*, 3743-3748.
17
18
19
20
21
22

23 (46) Bieschke, J.; Herbst, M.; Wiglenda, T.; Friedrich, R. P.; Boeddrich, A.; Schiele, F.;
24 Kleckers, D.; Lopez del Amo, J. M.; Grüning, B. A.; Wang, Q.; Schmidt, M. R.; Lurz, R.;
25 Anwyl, R.; Schnoeg, S.; Fändrich, M.; Frank, R. F.; Reif, B.; Günther, S.; Walsh, D. M.;
26 Wanker, E. E. Small-molecule conversion of toxic oligomers to nontoxic β -sheet-rich amyloid
27 fibrils. *Nat. Chem. Biol.* **2012**, *8*, 93-101.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
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