Results in Chemistry 3 (2021) 100230

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

Synthesis and biological evaluation of novel β-cyclodextrin-fluvastatin conjugates

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ARTICLE INFO

Keywords:
Antiaggregant
Cholesterol
Cyclodextrins
Cytotoxicity
Statin

ABSTRACT

Drugs that may regulate lipid metabolism and lower cholesterol, such as statins (drugs that reduce de novo cholesterol synthesis) or cyclodextrins (that promote cholesterol removal), are currently investigated as potential therapeutics for neuronal disorders like Alzheimer's and Niemann Pick type C disease. Fluvastatin is a member of the statin class widely used in preventing heart diseases as it lowers cholesterol and other lipids. β-cyclodextrin derivatives can also act as cholesterol scavengers to promote cholesterol efflux from cells to extracellular acceptors.

This context has inspired us to synthesize and characterize two new cyclodextrin-fluvastatin conjugates representing the first example of cyclodextrin conjugates of statins.

We synthesized 3- and 6-functionalized β-cyclodextrin with fluvastatin through an amide bond. We studied the stability, cytotoxicity and protective activity in cells of the new derivatives. We observed that the Fluvastatin cyclodextrin conjugates are stable in plasma and mouse brain homogenates. Moreover, we found that the fluvastain derivatives are well tolerated by cultured neuron cells, and they completely rescue from cell death induced by Aβ oligomers. Overall, the fluvastain derivatives have potential as therapeutic agents in diseases related to cholesterol dyshomeostasis.

Introduction

Statins are the first-line drugs for treating lipid disorders, preventing cerebrovascular diseases and atherosclerosis [1]. They can reduce cholesterol levels in plasma, by competitively blocking the active site of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase, a key enzyme of the endogenous cholesterol synthesis pathway. Some statins can also increase low-density lipoprotein (LDL) receptors on the membrane surface, thus enhancing the catabolism of cholesterol [2]. Among the class, three statins (lovastatin, simvastatin, and pravastatin) are derived from fungi and four statins (atorvastatin, rosuvastatin, fluvastatin, and pitavastatin) are synthetic [3]. Fluvastatin is widely prescribed for patients suffering from hypercholesterolemia and mixed dyslipidemia, in order to reduce endogenous cholesterol synthesis and to lower LDL-cholesterol, apolipoprotein B and triglycerides. Lately fluvastatin has also been prescribed to prevent cardiovascular events thanks to its capacity to enhance cholesterol clearance. Similarly to other statins fluvastatin is orally administered and it is metabolised by hepatic cytochromes in inactive metabolites [4].

Despite the widespread use and benefits of statins to lower cholesterol, some side effects have been reported: statin-associated muscle symptoms (SAMSs), which are the most observed, and new-onset of type 2 diabetes mellitus, whose mechanism are still unclear [5]. Fluvastatin also causes myopathy characterized by myalgia, muscular weakness and creatine kinase increase [4]. However, the side effects of fluvastatin are fewer than those observed for other statins.

Epidemiology studies have also shown that statins decrease the risk of Alzheimer's Disease (AD) [6,7]. Despite the literature is still

; Aβ, Amyloid beta; AD, Alzheimer's Disease; APP, Amyloid precursor protein; CD, Cyclodextrin; $CD6NH_2$, 6A-amino-6A-deoxy-β-cyclodextrin; $CD3NH_2$, 3A-amino-3A-deoxy-2A(S),3A(S)-β-cyclodextrin; DMTMM, Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride; Flu, Fluvastatin; HMG-CoA, 3- hydroxy-3-methylglutaryl-coenzyme A; MTT, Tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; NPC, Niemann Pick type C disease.

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https://doi.org/10.1016/j.rechem.2021.100230
Received 23 August 2021; Accepted 28 October 2021
Available online 6 November 2021

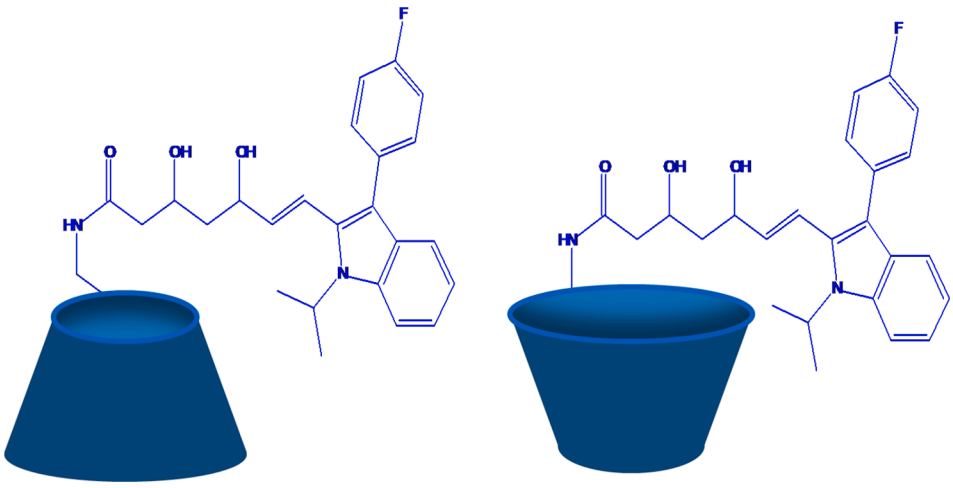

Fig. 1. Schematic of CD6Flu and CD3Flu.

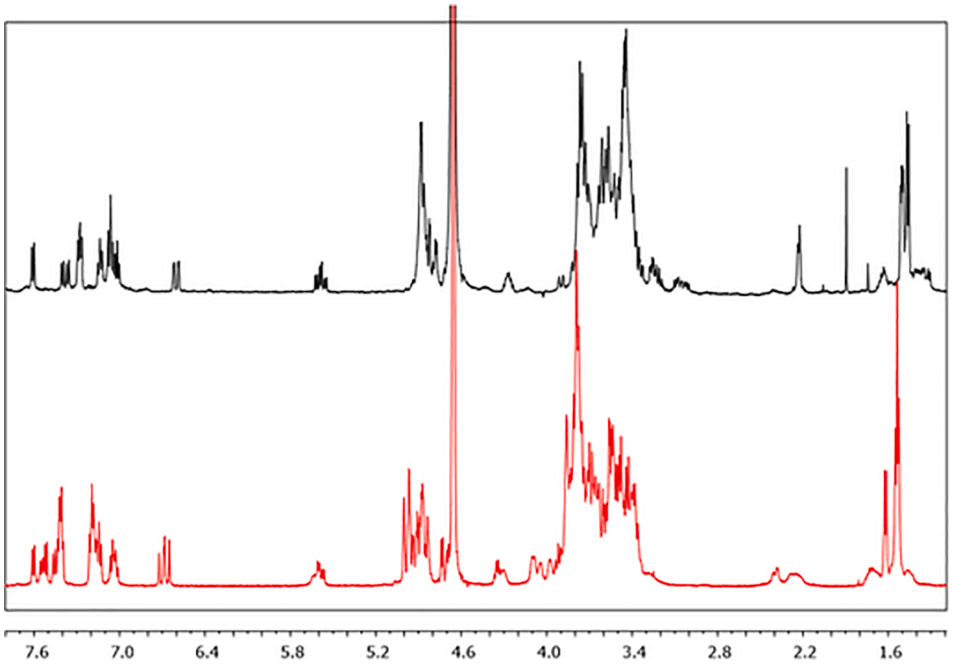

Fig. 2. ^{1}H NMR spectra of CD6Flu (upper) and CD3Flu (lower) (500 MHz, D_2O, 1 mM).

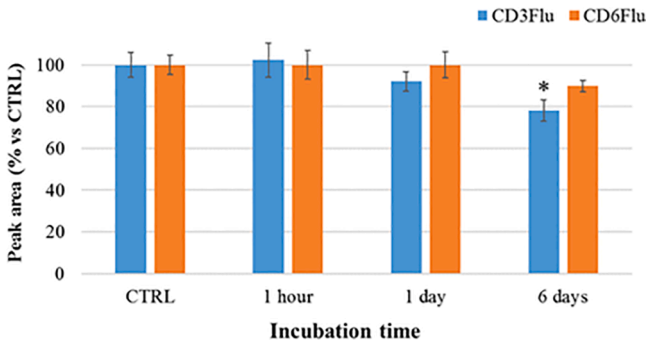

Fig. 3. Kinetic stability of CD3Flu and CD6Flu in human plasma at 37 °C for six days. The relative amounts of CDFlu were determined by HPLC analyses. Values are reported as Mean ± SD (n = 3, * $p < 0.05$ vs CTRL).

controversial on the role of cholesterol in AD, recent data point on cholesteryl esters (CE), the storage product of excess, and their effects on amyloid precursor protein (APP) processing, which regulates amyloid-β (Aβ) abundance and accumulation [8,13]. Growing evidence suggests a correlation between hypercholesterolemia, neurodegeneraion and cognitive decline [14,17].

Furthermore, high cholesterol levels in lysosomes and late endosomes have been found in several pathologies such as Niemann Pick type C disease (NPC) that is a rare lysosomal disorder caused by a genetic mutation in NPC1 or NPC2 gene [18]. These genes encode for proteins that regulate cholesterol transport from late endosomes-lysosomes to other intracellular compartments, such as the *trans*-Golgi network [18,19]. As a consequence, cholesterol and sphingolipids aberrantly accumulate inside lysosomes of neuronal cells [18]. The main sign of NPC rely on the liver, lungs, and the central nervous system, leading to neurodegeneration [20,22].

In recent years, cyclodextrins (CDs) [23], cyclic oligomers of α-1,4-D-glucose, have also been proposed for the removal of cholesterol as

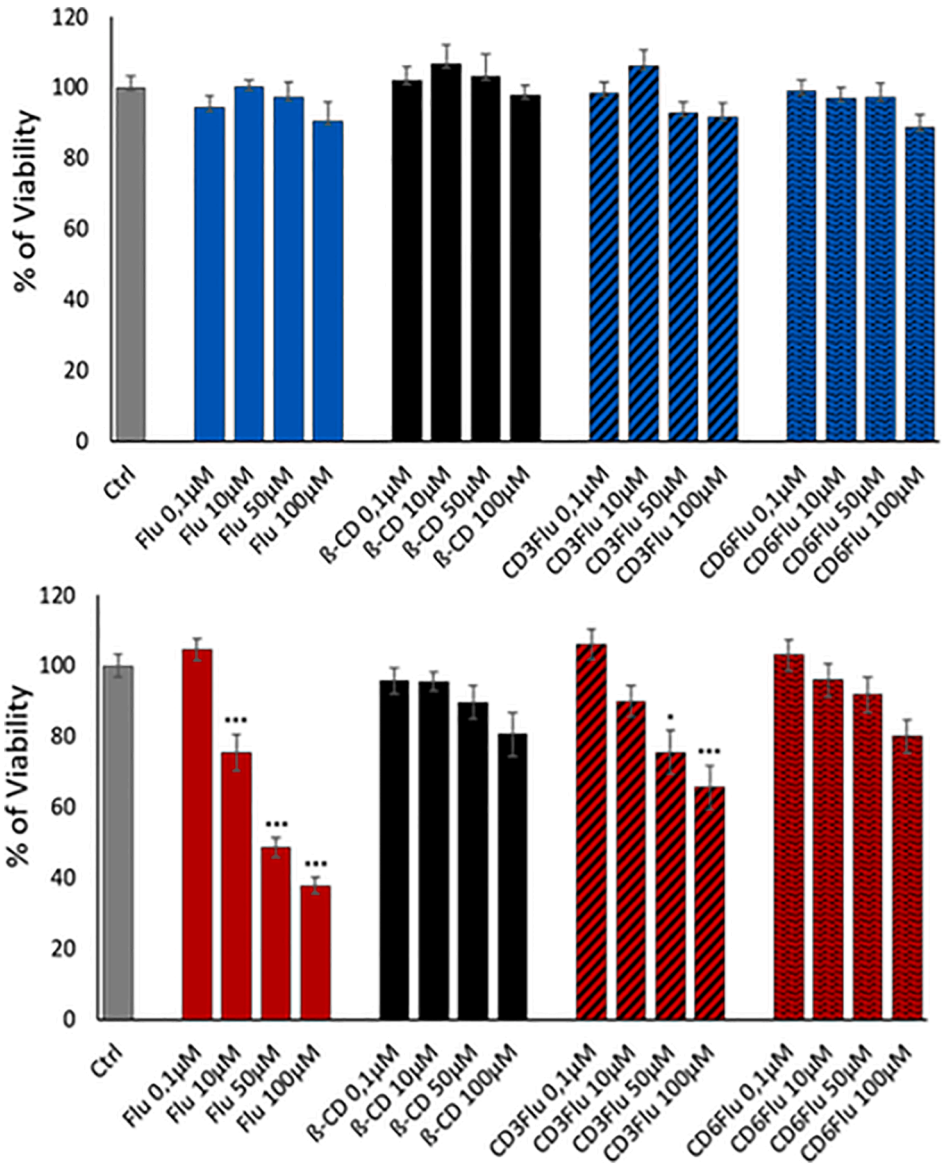

Fig. 4. MTT assay was performed on pure neuronal cultures after 24 h and 48 h treatments. Cells were exposed to increasing concentrations of CD3Flu and CD6Flu. Appropriate controls were also tested. Each graph represents three experiments with n = 3–5 each. Bars represent means ± SEM of three independent experiments with n = 3–5 each. ***P < 0.001, *P < 0.05vs Ctrl by One-Way ANOVA + Tukey Test.

therapeutic nanocavities [24]. β-CDs are able to include cholesterol better than other CDs [25,26]. Furthermore, CDs directly affect the cell membrane components. β-CDs modify the compositions of the lipid bilayer by extracting cholesterol through cell surface distribution [27] and can complex cholesterol in vitro Blood Brain Barrier model [28]. Moreover, in vivo experiments on transgenic mice demonstrated that hydroxypropyl-β-CD (HP-β-CD, Trappsol) [29] treatment reduced the atherosclerotic lesions due to a continuous cholesterol and lipid rich diet [30].

In vitro studies have also shown the capability of HP-β-CD to reduce stored cholesterol in primary neurons and astrocytes [31]. The intrathecal intake of HP-β-CD reduced the cholesterol storage in the transgenic mice model of NPC disease [32]. In 2009 Food Drug Administration approved the use of HP-β-CD to twin girls affected by NPC1 disease [33]. After few years, HP-β-CD was administrated intrathecally as the safest and most effective route to ameliorate the symptoms of NPC1 [34]. Trappsol is being evaluated in clinical trials for the treatment of NPC1 and for early Alzheimer's disease.

Moreover, CDs inhibit Aβ aggregation, which is considered one of the main pathological events occurring during AD progression [35–37]. Treatment with HP-β-CD lowered Aβ aggregation and improved memory in mice transgenic model of AD [38].

The biocompatibility and biodegradability of CDs make them nanocontainers widely used in pharmaceutical formulations and sequestering agents in the food and cosmetic industry [23,39,40]. Chemical modification of CDs has improved their properties for many applications [41–44]. A successful example is sugammadex [45], the first selective reversal agent for rocuronium approved in 2015 by FDA.

More recently, the interest in developing CD-drug conjugates has been growing [46–50]. CDs have been covalently modified with anti-inflammatory [48] and anticancer drugs [43]. The derivatization of the drug with CD can increase its water solubility and bioavailability significantly.

Based on the potential of CD derivatives, in this work, we present new β-CD conjugates of Fluvastatin (CDFlu), which could combine the pharmacological activity of Flu and the properties of the β-CD cavity (Fig. 1). Cyclodextrins have been used to prepare inclusion complexes of Flu, a poorly water soluble statin [51,52].

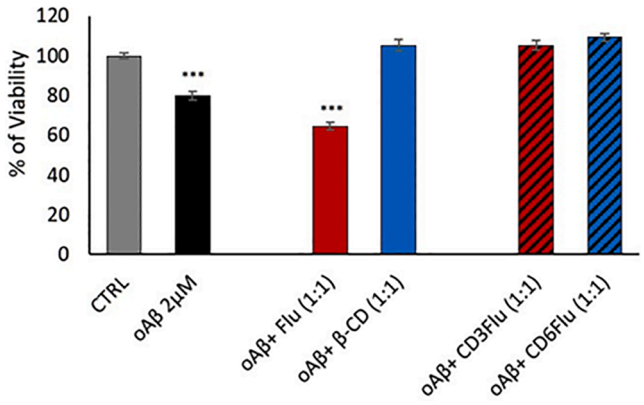

Fig. 5. MTT assay was performed on primary neuronal cultures after 48 h treatments. Cells were exposed to 2 μM Aβ oligomers incubated with or without CD3Flu, CD6Flu at the molar ratio of 1:1. Co-incubations with Flu and β-CD alone were also performed as experimental controls. Bars represent means ± SEM of two independent experiments with n = 3. ***P < 0.001 vs Ctrl by One-Way ANOVA + Tukey Test.

We found that the covalent linkage between CD and Flu brings new properties to the conjugate.

We studied the cytotoxicity of new derivatives and their stability in plasma and brain homogenates. We also investigated the protective ability of fluvastatin derivatives against cell death induced by Aβ oligomers compared to free fluvastatin. We found that CDFlu conjugates are stable in plasma and mouse brain homogenates. The functionalization of Flu with β-CD makes the conjugates less toxic than Flu (20% and 37 % respectively at 0.100 M) in cultured neuron cells. Moreover, while Flu seemed to potentiate Aβ toxicity, exposure to CDFlu showed a complete rescue from cell death.

Material and methods

Chemicals

Fluovastatin racemic mixture (+)-3R,5S and (-)-3S, 5R enantiomers (Flu, TCI), 3A-amino-3A-deoxy-2A(S),3A(S)-β-cyclodextrin ($CD3NH_2$, TCI), 6A-amino-6A-deoxy-β-cyclodextrin ($CD6NH_2$, Cyclolab), 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM, TCI) were used without further purification. TLC was carried out on silica gel plates (Merck 60-F254). Carbohydrate derivatives were detected on TLC with the anisaldehyde test. $Aβ_{1-40}$ (Aβ) was purchased from Bachem.

Synthesis of 3A-amino-3A-deoxy-2A(S),3A(S)-β-cyclodextrin-fluvastatin (CD3Flu)

$CD3NH_2$ (100 mg, 0.088 mmol), Flu (36.28 mg, 0.088 mmol) and DMTMM (24.40 mg, 0.088 mmol) were separately dissolved in 600 μl of water. 200 μl of $CD3NH_2$ and 200 μl of DMTMM were added to the Flu solution every 15 min. The reaction mixture was kept under stirring at room temperature for 24 h. The product was purified using flash chromatography with an Rp-C18 column, eluted with a linear gradient of H_2O-MeOH (0 → 100%). The product was collected and lyophilized. Yield: 29%. Rf = 0.38 (PrOH/AcOEt/H_2O/NH_3 5:3:2:1). MALDI-TOF: m/z = 1549.552 $[M + Na]^+$, m/z = 1565.521 $[M + K]^+$.

^{1}H NMR: (D_2O, 500 MHz) δ (/ppm): 7.60 (d, 1H, $J_{14,13}$ = 8.3 Hz, H-14 Flu of diastereomer 1), 7.55 (d, 1H, $J_{14,13}$ = 8.3 Hz, H-14 Flu of diastereomer 2), 7.52 (d, $J_{11,12}$ = 7.8 Hz, H-11 Flu of diastereomer 1), 7.46 (d, $J_{11,12}$ = 7.9 Hz, H-11 Flu of diastereomer 2), 7.41 (m, 2H, H −2′-H-6′ Flu), 7.19 (m, 3H, H-3′,H-5′ Flu and H-13 of diastereomer 1), 7.14 (t, 1H, H-13 of diastereomer 2), 7.05 (t, 1H, J = 7.7 Hz, H-12 Flu of diastereomer 1), 7.03 (t, 1H, J = 7.7 Hz, H-12 Flu of diastereomer 1), 6.71 (d, 1H, $J_{7,6}$ = 16.0 Hz, H-7 Flu of diastereomer 1), 6.67 (m, 1H, H-7 Flu of diastereomer 2), 5.72 (dd, 2H, H-6 Flu of diastereomer 1 and 2), 5.2–4.9 (m, 8H, H-1 CD and H-16 Flu), 4.47 (m, 1H, $J_{7,6}$ = 16.1 Hz H-5 Flu of diastereomer 2), 4.16 (m, 1H, H-3 CD), 4.10–3.4 (m, 43 H, H-5, H-6, H-3, H-2, H-4 CD and H-3 Flu), 2.39 (m, 1H, H-2a Flu), 2.26 (m, 1H, H-2b Flu), 1.71 (m, 1H, H-4a Flu), 1.65–1.49 (m, 6H, H-17 e H-18 Flu), 1.46 (m, 1H, H-4b Flu). Circular dichroism (H_2O) (λ nm, [Θ], deg × cm^2/dmol): 254 (−1.87), 328 (−0.090), 352 (0.014). UV–vis (λ nm, ε $M^{-1}cm^{-1}$): 238 (23836).

Synthesis of 6A-amino-6A-deoxy-β-cyclodextrin-fluvastatin (CD6Flu)

The derivative was synthesized and purified as CD3Flu, starting from $CD6NH_2$ instead of CD3NH2. Yield: 17%. Rf = 0.70 (PrOH/AcOEt/H_2O/NH_3 5:2:3:1). MALDI-TOF: m/z = 1549.546 $[M + Na]^+$, m/z = 1565.521 $[M + K]+$. ^{1}H NMR: (D_2O, 500 MHz). δ (/ppm): 7.61 (d, 1H, $J_{14,13}$ = 8.4 Hz, H-14 Flu), 7.40 (d, $J_{11,12}$ = 8.0 Hz, H-11 Flu of diastereomer 1), 7.36 (d, $J_{11,12}$ = 7.9 Hz, H-11 Flu of diastereomer 2), 7.28 (m, 2H, H −2′-H-6′ Flu), 7.14 (t, 1H, J = 7.5 Hz, H-13 Flu) 7.04 (m, 2H, H-3′-H5′ Flu), 7.02 (t, 1H, J = 7.3 Hz, H-12 Flu), 6.60 (d, 1H, $J_{7,6}$ = 16.0 Hz, H-7 Flu), 5.60 (dd, 1H, $J_{6,7}$ = 16.3 Hz, $J_{6,5}$ = 7.3 Hz H-6 Flu of diastereomer 2), 5.57 (dd, $J_{6,7}$ = 16.3 Hz, $J_{6,5}$ = 6.7 Hz H-6 Flu of diastereomer 2), 4.95–4.80 (m, 8H, H-1 CD), 4.79 (m, 1H, H-16 Flu), 4.26 (m, 1H, H-5 Flu), 3.90 (d, 1H, $J_{6,6'}$ = 4.0 Hz, H-6 CD of diastereomer 1), 3.85–3.30 (m, 38H, H-5, H-6, H-3, H-2, H-4 CD and H-3 Flu), 3.30–3.15 (m, 2H, H.4 CD), 3.08 (dd, 1H, $J_{6A,6'A}$ = 14.5 Hz, J6,5 = 9.0 Hz, H-6A of CD of diastereomer 1), 3.04 (dd, 1H, $J_{6A,6'A}$ = 14.4 Hz, J6,5 = 8.9 Hz, H-6A CD of diastereomer 2), 2.23 (m, 2H, H-2 Flu), 1.63 (m, 1H, H-4a Flu), 1.49 (m, 6H, H-17 and H-18 Flu) 1.35 (m, 1H, H-4b Flu). Circular dichroism (H_2O) (λ nm, [Θ], deg × cm^2/dmol): 258 (−0.76), 274 (−1.50), 325–0.58) 359 (0.095). UV–vis (λ nm, ε M^{-1} cm^{-1}): 233 (26446).

NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded at 25 °C with a Varian UNITY PLUS-500 spectrometer at 499.9 and 126 MHz, respectively. The 2D experiments (COSY, TOCSY, gHSQCAD, gHMBC, ROESY) were acquired by using 1000 data points, 256 increments, and a relaxation delay of 1.2 s. The spectra were referred to the solvent signal.

Mass spectrometry

Mass spectra were recorded on MALDI-TOF 5800 mass spectrometer (AB SCIEX, Foster City, CA). A saturated solution of 2,5-dihydroxybenzoic acid (DHB) in TA30 solvent (30:70 [v/v] acetonitrile:0.1% TFA in water) was used as the matrix. Nine spectra were acquired for each sample (three spots per sample and three spectra per spot).

UV–vis and Circular dichroism spectroscopy

UV–vis spectra were recorded on an Agilent 8452A diode array spectrophotometer. Circular dichroism measurements were performed on a JASCO spectropolarimeter (model J-1500).

Plasma and brain homogenate assay

3 mL of blood was treated with EDTA (5 mM) and centrifuged at 1200 g for 15 min. The supernatant plasma was diluted 1:1 with PBS, containing CD3Flu or CD6Flu (20 μM), and incubated at 37 °C for up to six days. The same protocol was used to assess the stability of the derivatives in brain homogenates from the transgenic mice model of AD, APPswe/PS1ΔE9, and the related wild type. In this case, the total incubation time was 21 h. All the samples were analysed by using an HPLC system (LC-20AP Shimadzu). The chromatographic analyses were performed with eluent A (0.1% TFA in water) and B (0.1% TFA in acetonitrile) on a C18 column (250 × 4.6 mm, Phenomenex) at a flow rate of 1 mL/min. The detection of the Flu derivatives was monitored at 300

nm. The use of brain homogenates was in accordance with the Institutional Guidelines.

Pure neuronal cultures

Cell viability: Cultures of pure cortical neurons were obtained from rats at embryonic day 15 as previously described. [53] Cortical cells were dissected, mechanically dissociated, and seeded in a Neurobasal Medium supplemented with B27 (Gibco, Thermofisher). Cortical cells were plated on 96-well plates precoated with 0.1 mg mL 1 poly-D-lysine and incubated at 37 °C with 5% CO_2 in a humidified atmosphere. Cytosine arabinoside (1-β-D-arabinofuranosylcytosine, Ara-C) (3–10 μM) was added to the cultures 18 h after plating to avoid the proliferation of non-neuronal elements and was kept for 3 days before medium replacement. To test the activity of CD3Flu and CD6Flu cells were exposed to 0.1, 10, 50 and 100 μM concentrations. Appropriate controls were also tested at the same concentrations.

Anti-oligomerization activity: Aβ oligomers were prepared as previously described [54]. Mature neurons were exposed for 48 h at 37 °C to the pre-incubated mix of oAβ alone or in combination with CD3Flu or CD6Flu (1:1 M ratio). To evaluate the ability of these conjugates to prevent Aβ oligomers formation and toxicity, we measured cell viability by MTT assay.

Results and discussion

Structural characterization

We performed the conjugation of β-CD with Flu by a condensation reaction of a mono-amino-CD (6A-amino-6A-deoxy-ß-cyclodextrin or 3A-amino-3A-deoxy-2A(S),3A(S)-β-cyclodextrin) and the carboxylic group of Flu (Fig. S1). DMTMM was used as the activating agent in an H_2O solution.

Derivatives were isolated as mixtures of diastereomers and were characterized by NMR spectroscopy and MALDI-TOF mass spectrometry. ^{1}H NMR spectra of the products (Fig. 2) were assigned using COSY, TOCSY, HSQC, HMBC, and ROESY (Figs. S2 and S11). The ^{1}H NMR spectra depend on the concentration. At higher concentration (>5 mM) the signals became broad (Fig. S12). This behaviour may suggest that the intermolecular interaction leads to the formation of aggregates at high concentrations. The two diastereomers obtained as a mixture from the condensation reaction with (±)Flu show different spectra. In the spectra of CD3Flu, Flu aromatic protons of both the diastereomers resonate between 8 and 7 ppm as assigned by 2D spectra. Moreover, the signals of H-7, H-6 and H-5 of Flu residue for each diastereomer can be assigned in the spectra.

The spectrum of CD6Flu shows the spreading of CD moiety's signal, particularly of the H-6 protons. The H-11, H-12 and H-6 of Flu and H-6A, H-5A and H-4A of CD moiety show different chemical shifts for each diastereomer.

ROESY spectra of CD3Flu and CD6Flu show correlations between all the aromatic protons of Flu and H-3, H-5 and H-6 protons of CD (Figs. S6 and S11).

As for CD6Flu, the cross-peaks are more intense for the protons of the F-benzene ring and the CD protons, in keeping with the inclusion of the benzene ring. As for CD3Flu also H-11, H-12, H-13 and H-14 show strong cross-correlations with the CD protons. Also, the methyl groups show cross-peaks with the CD protons.

Since the two compounds have the same molecular weight, the mass spectra show the peaks at the same *m/z* values; in particular, the spectra show two peaks, resulting from single-charged cation adducts of the Flu derivatives (*m/z* 1549 for [M + Na]+ and *m/z* 1565 for [M + K]+ (Fig. S13). The identity of the compounds is further proved by comparing the experimental isotope distribution and the theoretical one (Figs. S13 and S14).

Stability studies in plasma and brain homogenates

The biological stability of CD6Flu and CD3Flu was assessed both in human plasma and brain homogenates from the transgenic mice model of AD (APPswe/PS1ΔE9 and the related wild-type cells).

We found that the covalent linkage between the CD unit and Flu is stable in the biological environments used. Indeed, the amount of CD6Flu and CD3Flu does not significantly decrease during the incubation with human plasma within a day (Fig. 3). Such a trend is kept by CD6Flu up to six days, whereas the CD3Flu concentration slightly decreases at the end of the incubation period. Both CD6Flu and CD3Flu are also stable in the mice brain homogenates with 21 h of incubation (Fig. S15).

Toxicity studies in vitro

To evaluate the potential toxicity of the two conjugates, increasing concentrations (0.1, 10, 50 and 100 μM) were used and tested at two different times: 24 h and 48 h on primary cortical neurons. We used Flu and β-CD at the same conditions as experimental controls to evaluate the advantages of the conjugation.

After 24 h incubation, all treatments were devoid of toxicity. Longer exposure revealed that concentration of Flu higher than 10 μM produced neuronal death (50 μM = 48% and 100 μM = 37%) while β-CD showed a 20% toxicity only after treatment with the higher concentration used (100 μM). The functionalization of Flu with β-CD makes the conjugates less toxic compared to Flu, in particular, CD6Flu was safer than the isomer CD3Flu (Fig. 4).

Finally, to verify whether the newly conjugated molecules possess the ability of some β-CD derivatives to inhibit Aβ assembly, we tested the activity of CD3Flu and CD6Flu against Aβ oligomer toxicity (Fig. 5). For this purpose, we incubated freshly prepared solutions of 40 μM Aβ monomers in the presence or in the absence of each compound and their controls (β-CD or Flu alone) at the molar ratio of 1:1. All the mixtures were kept for 48 h at 4 °C, according to a well-established protocol to obtain toxic oligomeric species of Aβ [54].

After incubation, samples were added to primary neuronal cultures at the final concentration of 2 μM and maintained for 48 h at 37 °C and 5%CO_2, and cell viability was tested by MTT assay. As expected, oligomers produced a significant decrease in cell viability. Interestingly, while Flu seemed to potentiate Aβ toxicity, exposure to CD3Flu or CD6Flu showed a complete rescue from cell death. These data prove that the CD moiety linked to Flu confers interesting protective properties to the new compounds, as reported for other β-CD derivatives [55].

Conclusions

Neurological disorders affect millions of people worldwide. The main challenges the researchers face are focused on finding the molecular origins of these pathologies, as well as on developing multifunctional compounds that can cure and/or prevent the onset of these devastating disorders.

Cholesterol dyshomeostasis has been linked to the risk of developing cardiovascular, neurodegenerative and many other disorders. Both statins and cyclodextrins have been largely used to reduce cholesterol levels. Statins, such as fluvostatin, act as reversible competitive inhibitors of HMG-CoA Reductase (hydroxymethyl glutaryl coenzyme A reductase), while cyclodextrins act as cholesterol sequestering agents.

The two new cyclodextrins 3- or 6- functionalized with fluvastatin reported in this paper are water-soluble molecules, stable both in human plasma and brain homogenates from the transgenic mice model of AD. Unlike fluvastatin, they are both non-toxic for neuronal cells. Moreover, the functionalization of fluovastin with β-CD makes the conjugates able to rescue from cell death by Aβ oligomers, unlike fluovastatin, which seemed to potentiate Aβ toxicity. This data supports the importance of functionalization in improving cyclodextrin and fluvastatin properties.

Therefore, cyclodextrin-fluvastatin derivatives may have great potential in treating neurological disorders related to cholesterol dyshomeostasis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors thank Università degli Studi di Catania (Piano di incentivi per la ricerca di Ateneo 2020/2022 Pia.ce.ri.) for the financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2021.100230.

References

[1] R.S. Rosenson, Statins in atherosclerosis: Lipid-lowering agents with antioxidant capabilities, Atherosclerosis 173 (1) (2004) 1–12, https://doi.org/10.1016/S0021-9150(03)00239-9.
[2] A. Oesterle, U. Laufs, J.K. Liao, Pleiotropic effects of statins on the cardiovascular system, Circ. Res. 120 (1) (2017) 229–243, https://doi.org/10.1161/CIRCRESAHA.116.308537.
[3] B.B. Adhyaru, T.A. Jacobson, Safety and efficacy of statin therapy, Nat. Rev. Cardiol. 15 (12) (2018) 757–769, https://doi.org/10.1038/s41569-018-0098-5.
[4] E.N. Liberopoulos, S.S. Daskalopoulou, D.P. Mikhailidis, A.S. Wierzbicki, M. S. Elisaf, A review of the lipid-related effects of fluvastatin, Curr. Med. Res. Opin. 21 (2) (2005) 231–243, https://doi.org/10.1185/030079905X26261.
[5] W.-T. Liu, C. Lin, M.-C. Tsai, C.-C. Cheng, S.-J. Chen, J.-T. Liou, W.-S. Lin, S.-M. Cheng, C.-S. Lin, T.-P. Tsao, Effects of pitavastatin, atorvastatin, and rosuvastatin on the risk of new-onset diabetes mellitus: a single-center cohort study, Biomedicines 8 (11) (2020) 499, https://doi.org/10.3390/biomedicines8110499.
[6] V.F. Langness, R. van der Kant, U. Das, L. Wang, R.D.S. Chaves, L.S.B. Goldstein, J. Olzmann, Cholesterol-lowering drugs reduce APP processing to Aβ by inducing APP dimerization, Mol. Biol. Cell 32 (3) (2021) 247–259, https://doi.org/10.1091/mbc.E20-05-0345.
[7] A. Fracassi, M. Marangoni, P. Rosso, V. Pallottini, M. Fioramonti, S. Siteni, M. Segatto, Statins and the brain: more than lipid lowering agents? Curr. Neuropharmacol. 17 (1) (2018) 59–83, https://doi.org/10.2174/1570159X15666170703101816.
[8] D.L. Sparks, T.A. Martin, D.R. Gross, J.C. Hunsaker, Link between heart disease, cholesterol, and Alzheimer's disease: a review, Microsc. Res. Tech. 50 (4) (2000) 287–290, https://doi.org/10.1002/1097-0029(20000815)50:4<287::AID-JEMT7>3.0.CO;2-L.
[9] J.M. Dietschy, S.D. Turley, Cholesterol metabolism in the central nervous system during early development and in the mature animal, J. Lipid Res. 45 (8) (2004) 1375–1397, https://doi.org/10.1194/jlr.R400004-JLR200.
[10] S.M. Ostrowski, B.L. Wilkinson, T.E. Golde, G. Landreth, Statins reduce amyloid-β production through inhibition of protein isoprenylation, J. Biol. Chem. 282 (37) (2007) 26832–26844, https://doi.org/10.1074/jbc.M702640200.
[11] C.-S. Chu, P.-T. Tseng, B. Stubbs, T.-Y. Chen, C.-H. Tang, D.-J. Li, W.-C. Yang, Y.-W. Chen, C.-K. Wu, N. Veronese, A.F. Carvalho, B.S. Fernandes, N. Herrmann, P.-Y. Lin, Use of statins and the risk of dementia and mild cognitive impairment: a systematic review and meta-analysis, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-24248-8.
[12] H. Jick, G.L. Zornberg, S.S. Jick, S. Seshadri, D.A. Drachman, Statins and the risk of dementia, Lancet 356 (9242) (2000) 1627–1631, https://doi.org/10.1016/S0140-6736(00)03155-X.
[13] F.M. Feringa, R. van der Kant, Cholesterol and Alzheimer's disease; from risk genes to pathological effects, Front. Aging Neurosci. (2021) 333, https://doi.org/10.3389/FNAGI.2021.690372.
[14] G.P. Jarvik, E.M. Wijsman, W.A. Kukull, G.D. Schellenberg, C. Yu, E.B. Larson, Interactions of apolipoprotein e genotype, total cholesterol level, age, and sex in prediction of alzheimer's disease: a case-control study, Neurology 45 (6) (1995) 1092–1096, https://doi.org/10.1212/WNL.45.6.1092.
[15] M. Kivipelto, A. Solomon, Cholesterol as a risk factor for Alzheimer's disease – epidemiological evidence, Acta Neurol. Scand. 114 (s185) (2006) 50–57, https://doi.org/10.1111/j.1600-0404.2006.00685.x.
[16] C. Ma, Z. Yin, P. Zhu, J. Luo, X. Shi, X. Gao, Blood cholesterol in late-life and cognitive decline: a longitudinal study of the Chinese elderly, Mol. Neurodegener. 2017 121 12 (1) (2017) 1–9, https://doi.org/10.1186/S13024-017-0167-Y.
[17] R. Loera-Valencia, M.-A.-M. Ismail, J. Goikolea, et al., Hypercholesterolemia and 27-Hydroxycholesterol Increase S100A8 and RAGE Expression in the Brain: a Link Between Cholesterol, Alarmins, and Neurodegeneration, Mol. Neurobiol. 2021 (1) (2021) 1–14, https://doi.org/10.1007/S12035-021-02521-8.
[18] C. Tranchant, Niemann-Pick disease type C, Prat. Neurol. - FMC 2 (4) (2011) 229–236, https://doi.org/10.1016/j.praneu.2011.07.001.
[19] D.S. Ory, Niemann-Pick type C: a disorder of cellular cholesterol trafficking, Biochim. Biophys. Acta – Mol. Cell Biol. Lipids 1529 (1–3) (2000) 331–339, https://doi.org/10.1016/S1388-1981(00)00158-X.
[20] M.C. Patterson, A riddle wrapped in a mystery: understanding Niemann-Pick disease, Type C, Neurologist 9 (6) (2003) 301–310, https://doi.org/10.1097/01.nrl.0000094627.78754.5b.
[21] T. Manabe, T. Yamane, T. Higashi, P.G. Pentchev, K. Suzuki, Ultrastructural changes in the lung in Niemann-Pick type C mouse, Virchows Arch. 427 (1) (1995) 77–83, https://doi.org/10.1007/BF00203741.
[22] M.T. Vanier, G. Millat, Niemann-Pick disease type C, Clin. Genet. 64 (4) (2003) 269–281, https://doi.org/10.1034/j.1399-0004.2003.00147.x.
[23] P. Jansook, N. Ogawa, T. Loftsson, Cyclodextrins: structure, physicochemical properties and pharmaceutical applications, Int. J. Pharm. 535 (1–2) (2018) 272–284, https://doi.org/10.1016/j.ijpharm.2017.11.018.
[24] M. Mahjoubin-Tehran, P.T. Kovanen, S. Xu, T. Jamialahmadi, A. Sahebkar, Cyclodextrins: Potential therapeutics against atherosclerosis, Pharmacol. Ther. 214 (2020) 107620, https://doi.org/10.1016/j.pharmthera.2020.107620.
[25] V.M. Atger, M.M. De La Llera, G.W. Stoudt, W.V. Rodrigueza, M.C. Phillips, G. H. Rothblat, Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells, J. Clin. Invest. 99 (4) (1997) 773–780, https://doi.org/10.1172/JCI119223.
[26] E.P.C. Kilsdonk, P.G. Yancey, G.W. Stoudt, F.W. Bangerter, W.J. Johnson, M. C. Phillips, G.H. Rothblat, Cellular cholesterol efflux mediated by cyclodextrins, J. Biol. Chem. 270 (29) (1995) 17250–17256, https://doi.org/10.1074/jbc.270.29.17250.
[27] T. Irie, K. Uekama, Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation, J. Pharm. Sci. 86 (2) (1997) 147–162, https://doi.org/10.1021/js960213f.
[28] V. Monnaert, S. Tilloy, H. Bricout, L. Fenart, R. Cecchelli, E. Monflier, Behavior of α-, β-, and γ-cyclodextrins and their derivatives on an in vitro model of blood-brain barrier, J. Pharmacol. Exp. Ther. 310 (2) (2004) 745–751, https://doi.org/10.1124/jpet.104.067512.
[29] M. Malanga, J. Szemán, É. Fenyvesi, I. Puskás, K. Csabai, G. Gyémánt, F. Fenyvesi, L. Szente, "Back to the Future'': a New look at hydroxypropyl beta-cyclodextrins, Pharmaceutics 105 (9) (2016) 2921–2931, https://doi.org/10.1016/j.xphs.2016.04.034.
[30] S. Zimmer, A. Grebe, S.S. Bakke, et al., Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming, Sci. Transl. Med. 8 (333) (2016), https://doi.org/10.1126/scitranslmed.aad6100.
[31] K.B. Peake, J.E. Vance, Normalization of cholesterol homeostasis by 2-hydroxypropyl-β- cyclodextrin in neurons and glia from Niemann-Pick C1 (NPC1)-deficient mice, J. Biol. Chem. 287 (12) (2012) 9290–9298, https://doi.org/10.1074/jbc.M111.326405.
[32] F.W. Chen, C. Li, Y.A. Ioannou, M.A. Deli, Cyclodextrin induces calcium-dependent lysosomal exocytosis, PLoS ONE 5 (11) (2010) 1–12, https://doi.org/10.1371/journal.pone.0015054.
[33] P. Calias, 2-Hydroxypropyl-β-cyclodextrins and the blood-brain barrier: considerations for Niemann-Pick Disease Type C1, Curr. Pharm. Des. 23 (40) (2018) 6231–6238, https://doi.org/10.2174/1381612823666171019164220.
[34] E. Berry-Kravis, J. Chin, A. Hoffmann, et al., Long-term treatment of Niemann-Pick Type C1 disease with intrathecal 2-hydroxypropyl-β-cyclodextrin, Pediatr. Neurol. 80 (2018) 24–34, https://doi.org/10.1016/j.pediatrneurol.2017.12.014.
[35] P. Camilleri, N.J. Haskins, D.R. Hewlett, β-Cyclodextrin interacts with the Alzheimer amyloid β-A4 peptide, FEBS Lett. 341 (2–3) (1994) 256–258, https://doi.org/10.1016/0014-5793(94)80467-2.
[36] A. Wahlström, R. Cukalevski, J. Danielsson, et al., Specific binding of a β-cyclodextrin dimer to the amyloid β peptide modulates the peptide aggregation process, Biochemistry 51 (21) (2012) 4280–4289, https://doi.org/10.1021/bi300341j.
[37] J. Danielsson, J. Jarvet, P. Damberg, A. Gräslund, Two-site binding of β-cyclodextrin to the Alzheimer Aβ(1–40) peptide measured with combined PFG-NMR diffusion and induced chemical shifts, Biochemistry 43 (20) (2004) 6261–6269, https://doi.org/10.1021/bi036254p.
[38] J. Yao, D. Ho, N.Y. Calingasan, N.H. Pipalia, M.T. Lin, F.F. Beal, Neuroprotection by cyclodextrin in cell and mouse models of Alzheimer disease, J. Exp. Med. 209 (13) (2012) 2501–2513, https://doi.org/10.1084/jem.20121239.
[39] A.C. Santos, D. Costa, L. Ferreira, et al., Cyclodextrin-based delivery systems for in vivo-tested anticancer therapies, Drug Deliv. Transl. Res. 11 (1) (2021) 49–71, https://doi.org/10.1007/s13346-020-00778-5.
[40] G. Crini, S. Fourmentin, É. Fenyvesi, G. Torri, M. Fourmentin, N. Morin-Crini, Cyclodextrins, from molecules to applications, Environ. Chem. Lett. 16 (4) (2018) 1361–1375, https://doi.org/10.1007/s10311-018-0763-2.
[41] L. Jicsinszky, G. Cravotto, Toward a Greener World—cyclodextrin derivatization by mechanochemistry, Mol 26 (17) (2021) 5193, https://doi.org/10.3390/MOLECULES26175193.
[42] A. Matencio, G. Hoti, Y.K. Monfared, et al., Cyclodextrin monomers and polymers for drug activity enhancement, Polymers (Basel) 13 (11) (2021) 1684, https://doi.org/10.3390/polym13111684.

[43] B. Tian, Y. Liu, J. Liu, Cyclodextrin as a magic switch in covalent and non-covalent anticancer drug release systems, Carbohydr. Polym. 242 (2020) 116401, https://doi.org/10.1016/j.carbpol.2020.116401.
[44] A. Kulkarni, P. Caporali, A. Dolas, et al., Linear cyclodextrin polymer prodrugs as novel therapeutics for Niemann-Pick Type C1 disorder, Sci. Rep. 8 (1) (2018), https://doi.org/10.1038/s41598-018-27926-9.
[45] M. Naguib, Sugammadex: another milestone in clinical neuromuscular pharmacology, Anesth. Analg. 104 (3) (2007) 575–581, https://doi.org/10.1213/01.ane.0000244594.63318.fc.
[46] M. Ceborska, Folate appended cyclodextrins for drug, DNA, and siRNA delivery, Eur. J. Pharm. Biopharm. 120 (2017) 133–145, https://doi.org/10.1016/j.ejpb.2017.09.005.
[47] D.M. George, R.J. Huntley, K. Cusack, et al., Prodrugs for colon-restricted delivery: Design, synthesis, and in vivo evaluation of colony stimulating factor 1 receptor (CSF1R) inhibitors, PLoS One. 13 (9) (2018), https://doi.org/10.1371/journal.pone.0203567 e0203567 Andrade PB, ed.
[48] H.M. Chu, R.X. Zhang, Q. Huang, C.C. Bai, Z.Z. Wang, Chemical conjugation with cyclodextrins as a versatile tool for drug delivery Conjugates of non-steroidal anti-inflammatory drugs with cyclodextrins, J. Incl. Phenom. Macrocycl. Chem. 89 (1-2) (2017) 29–38, https://doi.org/10.1007/s10847-017-0743-3.
[49] V. Oliveri, G. Vecchio, Metallocyclodextrins in medicinal chemistry, Future Med. Chem. 10 (6) (2018) 663–677, https://doi.org/10.4155/fmc-2017-0249.
[50] C. Yan, N. Liang, Q. Li, P. Yan, S. Sun, Biotin and arginine modified hydroxypropyl-β-cyclodextrin nanoparticles as novel drug delivery systems for paclitaxel, Carbohydr. Polym. 216 (2019) 129–139, https://doi.org/10.1016/j.carbpol.2019.04.024.
[51] S.M. Ali, S.K. Upadhyay, A. Maheshwari, M. Koketsu, Complexation of fluvastatin sodium with β-cyclodextrin: NMR spectroscopic study in solution, J. Incl. Phenom. Macrocycl. Chem. 55 (3–4) (2006) 325–328, https://doi.org/10.1007/s10847-006-9099-9.
[52] L. Jun, Y. Min, X. Wen-Rong, Enhanced oral bioavailability of fluvastatin by using nanosuspensions containing cyclodextrin, Drug Des. Dev. Ther. 12 (2018) 3491–3499, https://doi.org/10.2147/DDDT.S177316.
[53] M.L. Giuffrida, F. Caraci, B. Pignataro, et al., β-amyloid monomers are neuroprotective, J. Neurosci. 29 (34) (2009) 10582–10587, https://doi.org/10.1523/JNEUROSCI.1736-09.2009.
[54] M.P. Lambert, A.K. Barlow, B.A. Chromy, et al., Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins, Proc. Natl. Acad. Sci. U. S. A. 95 (11) (1998) 6448–6453, https://doi.org/10.1073/pnas.95.11.6448.
[55] V. Oliveri, S. Zimbone, M.L. Giuffrida, F. Bellia, M.F. Tomasello, G. Vecchio, Porphyrin cyclodextrin conjugates modulate amyloid beta peptide aggregation and cytotoxicity, Chem - A Eur J. 24 (24) (2018) 6349–6353, https://doi.org/10.1002/chem.201800807.