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'Clickable' 2,5-diketopiperazines as scaffolds for ligation of biomolecules: their use in Aβ inhibitor assembly†

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The synthesis of 1,3,6-trisubstituted-2,5-diketopiperazine scaffolds bearing up to three 'clickable' sites for further oxime bond or alkyne–azide cycloaddition ligations is described. The orthogonally Boc/Alloc protected DKP precursors prepared from L-lysine residues and an aminohexyl arm are efficiently prepared on a gram scale by sequentially using Fukuyama–Mitsunobu alkylation, dipeptide coupling and diketopiperazine ring formation as key steps. These scaffolds, with their glyoxylyl, aminooxy, alkynyl or azido functions, are "ready-to-use" platforms for biomolecular assembly. Their potentiality in this field was proved through the chemoselective ligation of Aβ-binding motifs, the KLVFFA peptide and the curcumin molecule. The inhibitory effect of these conjugates on Aβ amyloid fibril formation is reported using thioflavin T fluorescence assays and AFM observation.

Introduction

2,5-Diketopiperazines (DKPs), derived from the condensation of two α-amino acids with functionalized side chains, are attractive scaffolds for molecular assembly design and have been extensively used for the rational design of drugs and peptidomimetics.[1,2] DKPs in which additional functional groups are introduced at the lactam nitrogen positions can display up to four functionalities in a well-defined spatial manner. In this context, DKPs bearing functionalities such as an amine (*e.g.* derived from Lys or Orn) and/or a carboxylic acid (*e.g.* derived from Asp or Glu) are of particular interest. Considering the variety of orthogonal protecting groups for amines and the chemical orthogonality between amine and carboxylic acid functions, they can be regioselectively and diversely addressed. G. Gellerman *et al.* have used this strategy to construct a DKP library for combinatorial chemistry.[3,4] Orthogonal chemical reactions can also be used to modify a DKP core. One example has been reported by M. Tullberg *et al.* with a DKP scaffold bearing reaction sites for hydroxylation, Heck reaction, and Huisgen cycloaddition.[5] However, to the best of our knowledge, no 'ready-to-use' DKP for chemoselective ligation of biomolecules has been reported.[6] Our research group being interested in this field,[7] we focused on such DKPs (Fig. 1). We prepared then a series of DKPs allowing oxime bond or alkyne–azide cycloaddition ligations, which are commonly used for the conjugation of biomolecules such as peptides.[8]

We chose a cyclo(Lys–Lys) as a scaffold to benefit from the chemically addressable amino side chains and then easily introduced diverse 'clickable' functionalities. In addition, the use of natural lysines as starting materials is a way to control the spatial orientation of the conjugated molecules in a *cis*

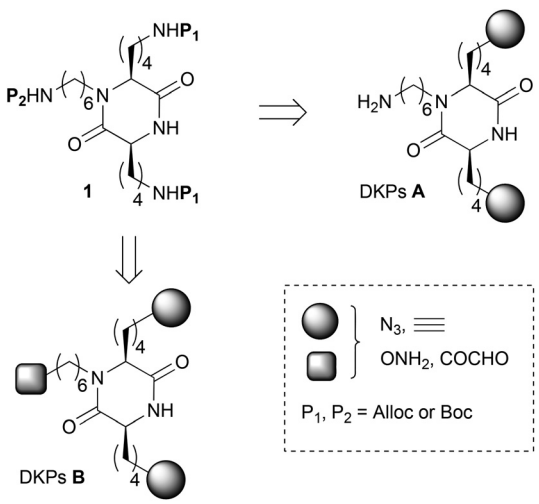

Fig. 1 Chemoselective addressable DKPs targeted in this study.

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†Electronic supplementary information (ESI) available: Kinetics of $A\beta_{40}$ fibril formation in the presence of DKPs **1b**, **18**, **20** and **21**, copies of ^{1}H and ^{13}C NMR spectra, and copies of MS and HRMS. See DOI: 10.1039/c4ob00541d

position. The 'clickable' DKPs such as DKPs **A** can be used to ligate two copies of the selected biomolecule possessing the complementary 'clickable' functionality. The aminohexyl arm at the N1-position of DKPs **A** is used to improve the solubility in the aqueous media in which are usually performed chemoselective ligation of biomolecules. This amino arm may also be used as an additional orthogonally 'clickable' site, as shown in DKPs **B**, to introduce a detecting agent or other functionalities depending on the desired application.

In this paper, we report the synthesis of these new DKPs *via* an orthogonally protecting group strategy from DKPs **1** as precursors (Fig. 1). We also investigate, through the ligation of well-known Aβ-binding motifs (the KLVFFA peptide and the curcumin molecule),[9] their potentiality as scaffolds in Aβ inhibitor assembly.

Results and discussion

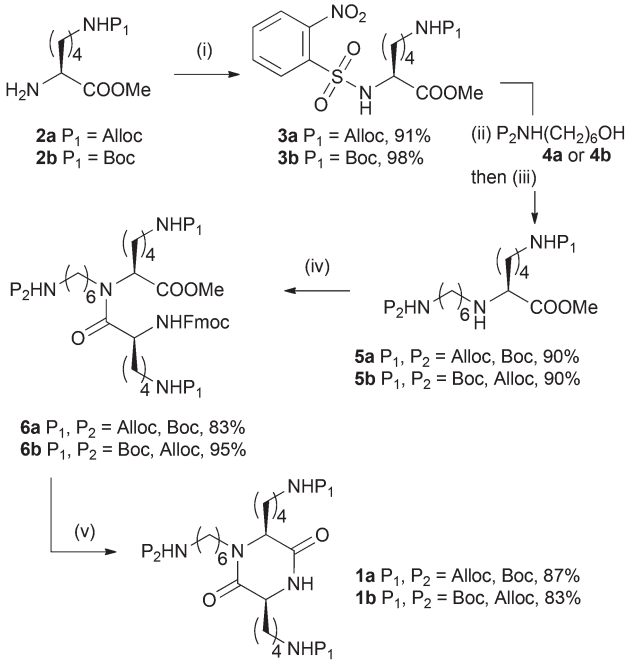
Synthesis of the DKP precursors

We first prepared the orthogonally protected DKPs **1a** and **1b** (P_1, P_2 = Boc or Alloc, Scheme 1). The strategy used is solution-phase synthesis, *i.e.* Nα-alkylation of the first lysine partner (**2a** or **2b**), condensation in solution of this Nα-alkylated Nε-protected aminoester (**5a** or **5b**) with the second Nα,ε-protected lysine partner, and then intramolecular cyclization of the resulting dipeptide. This strategy has been proved for the synthesis of such head-to-tail DKPs.[1,3,4,10,11] Very similar DKP scaffolds resulting from the condensation of two orthogonally protected lysine residues with Alloc, CBz or Fmoc groups and bearing a carboxymethyl group in the N1-position have been reported.[3a] In this case, as in most cases, reductive amination using aldehydes (*e.g.* glyoxylic aldehyde) is employed for Nα-alkylation of the first amino acid partner. In our case, a Fukuyama–Mitsunobu reaction[12,13] is applied for the Nα-monoalkylation of the first lysine partner using hydroxyl derivatives instead of aldehydes as alkylating agents. The nitrobenzenesulfonyl (Ns) group used as both a protecting and an activating group on the primary amines ensures the conversion into the secondary amines and prevents the formation of the tertiary amines often produced as by-products under reductive amination conditions with no sterically hindered agents.

Thus, the easily prepared Nε-Alloc protected lysine methyl ester **2a** was first protected as 2-nitrobenzenesulfonamide with a slight excess of 2-nitrobenzenesulfonyl chloride in the presence of Et_3N. The sulphonamide intermediate **3a**, which was obtained in 91% yield, was then alkylated under the Mitsunobu conditions (DIAD, PPh_3) using the 6-aminohexanol derivative, $BocHN(CH_2)_6OH$. After removal of the Ns group *via* aromatic nucleophilic substitution by thiophenol in the presence of K_2CO_3, the Nα-alkylated lysine aminoester **5a** was obtained in 90% yield from **3a** after chromatography purification. The same sequence was applied to the commercially available Nε-Boc protected lysine aminoester **2b** using $AllocHN(CH_2)_6OH$ as an alkylating agent to obtain **5b** with a similar yield (88% from **2b**).

In the literature, Nα-Boc protected amino acids are often used as the second partners. The cyclization is then realized under basic conditions after removal of the Nα-Boc group under acidic conditions. We chose to use commercially available Nα-Fmoc protected lysine residues. The coupling of the Fmoc-Lys(Alloc)-OH to the Nα-alkylated lysine aminoester **5a** was carried out using HATU as a coupling agent, which is known to be efficient for coupling of secondary amines. The dipeptide **6a** was thus efficiently obtained in 83% yield. Finally, removal of the Fmoc group and intramolecular cyclization of **6a** were realized in one pot using a mixture of piperidine in dichloromethane[14] to afford the DKP precursor **1a** in 87% yield. The DKP **1b** was obtained in the same manner from Fmoc-Lys(Boc)-OH and **5b** in 78% yield (two steps).

The strategy developed yielded the orthogonally protected DKPs **1a** and **1b** on a gram scale in more than 60% overall yield. The protected amino alcohol arms **4a** and **4b**, used to introduce the additional functionality into the N1-position of the DKP core, were easily prepared from the commercially available 6-aminohexanol and the corresponding chloroformates. Starting from commercially available or readily prepared amino acids, this approach may be applied to the synthesis of other orthogonally protected DKPs with Dde or CBz groups. In addition, by using Fmoc-Lys(Dde)-OH as the second lysine partner from **5a** or **5b**, DKPs with three orthogonally protecting sites can be also obtained.



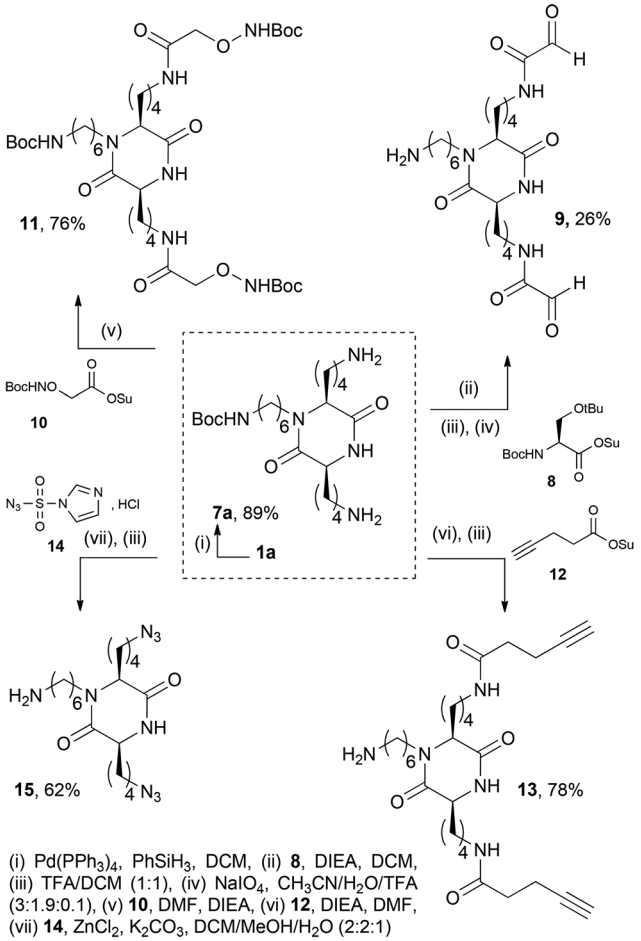
(i) Ns-Cl, Et_3N, H_2O/dioxane (1:1), (ii) $P_2NH(CH_2)_6OH$ (**4a** P_2 = Boc, **4b** P_2 = Alloc), PPh_3, DIAD, THF, (iii) PhSH, K_2CO_3, DMF, (iv) Fmoc-Lys(P_1)-OH (P_1 = Alloc from **5a**, P_1 = Boc from **5b**), HATU, DIEA, DCM, (v) piperidine/DCM (1:4)

Scheme 1 Synthesis of the orthogonally protected DKP precursors **1**.

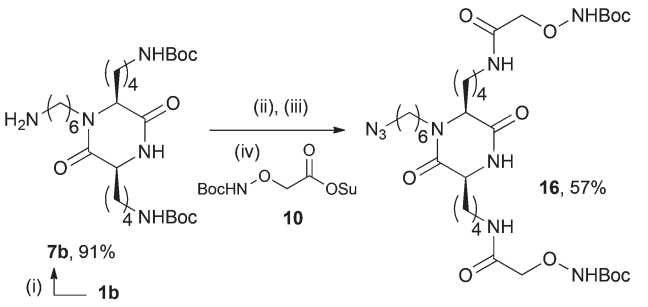
Synthesis of the 'clickable' DKPs

The orthogonally protected DKP **1a** was used as the common precursor of the 'clickable' DKPs **9**, **11**, **13**, **15** bearing glyoxylyl, aminooxy, alkynyl, or azido functions, respectively (Scheme 2). Except for **15**, these functions were easily introduced by N-acylation of the Alloc deprotected intermediate **7a**[15] using the activated carboxylic acids **8**, **10** and **12**. We preferred to use *N*-hydroxysuccimide esters as 'ready-to-use' activated species but the formation *in situ* of the activated species can also been realized.

In **9**, the glyoxylyl functions were introduced by coupling the Boc-Ser(*t*Bu)-OSu *N*-hydroxysuccinimide ester **8** on the free lysine residues of **7a**. The Boc-Ser(*t*Bu)-OH residue is commonly used as a masked glyoxylic acid equivalent in peptide chemistry to attach glyoxylyl groups to the N-terminus or to Nε-lysine residues of peptides. Indeed, after removal of the Boc and *t*Bu groups, periodate oxidation of the serine residue is a simple way to obtain the desired α-oxo aldehyde group.[16] The DKP **9** was then obtained in 26% overall yield (three steps). Loss of product during the last RP-HPLC purification step (only 42% yield) accounts for this modest yield, while the HPLC analysis of the oxidation reaction mixture shows a total conversion to **9**.



Scheme 2 Synthesis of the 'clickable' DKPs from **1a**.



Scheme 3 Synthesis of an orthogonally 'clickable' DKP from **1b**.

For DKP **11**, the aminooxy functions were incorporated as Boc-protected aminooxyacetyl ester **10**[17] with 76% yield. We preferred for storage keeping the protecting groups due to the high reactivity of the free aminooxy functions towards electrophilic agents. Oxime ligation being performed under acidic conditions in the presence of aldehydes, removal of the Boc acid sensitive protecting groups of **11** will be accompanied by *in situ* ligation reactions. The DKP **13** was similarly obtained from **7a** and 4-pentynoic acid succinimidyl ester **12**, after Boc removal of its *N*-aminohexyl arm, in 78% yield after purification.

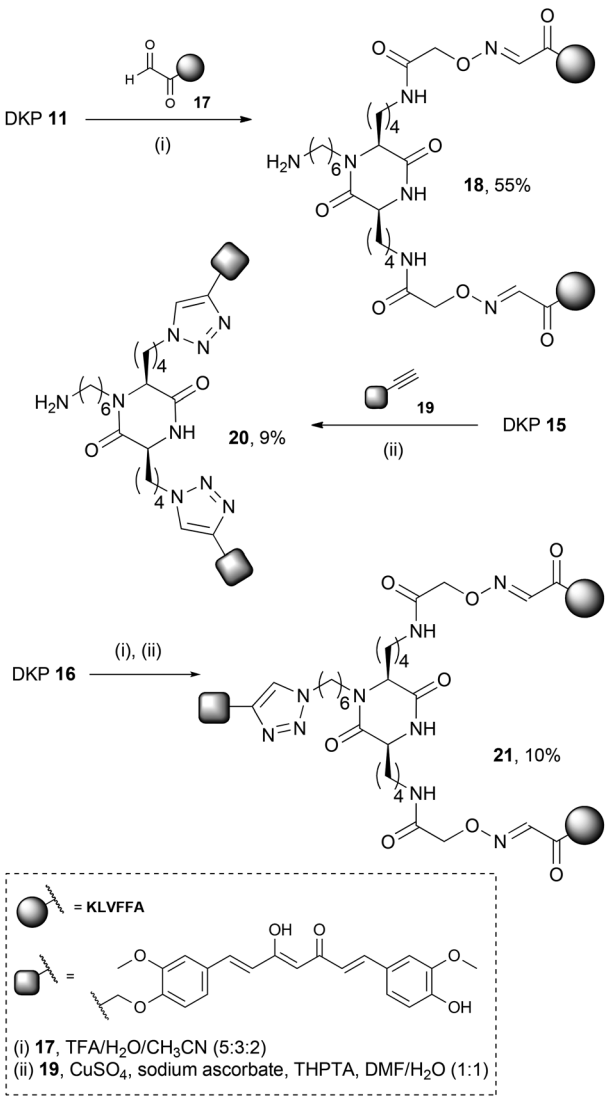
Finally, DKP **15** bearing azido functions was efficiently obtained (62% yield) by converting the free amino lysine residues of **7a** into azides using the hydrogen chloride salt of imidazole-1-sulfonyl azide **14** as a stable diazotransfer reagent,[18,19] followed by Boc removal.

The DKP **1b** was employed to access an orthogonally 'clickable' DKP that presents one azido group and two aminooxy precursors (compound **16**, Scheme 3).

Removal of the Alloc group of **1b** was performed, as for **1a**, using the $Pd^0/PhSiH_3$ [15] procedure, affording the DKP **7b** in more than satisfactory yield (91%). The free amino function of **7b** was then converted into azide as mentioned above for **15**. Then, the aminooxy functions were introduced, as Boc-aminooxyacetic acid succinimide ester (compound **10**), on the lysine side chains after removal of their Nε-Boc protecting groups. The DKP **16** was obtained in three steps with 57% yield. It is worth noting that the same strategy may be applied for **7a** to obtain the DKP bearing one aminooxy and two azido groups tethered on the lysine side chains.

Aβ inhibitor assembly

Synthesis. The 'clickable' DKPs **11**, **15** and **16** were applied in biomolecular assembly design through the ligation of Aβ-binding motifs, the KLVFFA peptide and the curcumin molecule (Scheme 4). Our research group is interested in the field of Alzheimer's disease inhibitors. In a previous study, we



Scheme 4 Chemoselective ligation of Aβ inhibitors.

showed that ligation of two copies of KLVFFA or curcumin on a cyclodecapeptide scaffold leads to more potent inhibitors than the Aβ-binding motifs alone.[20] Built to present at least two ligation sites, we were interested in evaluating our DKPs in this field.

To assemble the KLVFFA sequence, we chose the oxime bond ligation method from the DKP **11** that presents two masked aminooxy sites. The KLVFFA sequence bearing as a complementary function a glyoxylyl group at its N-terminus was first prepared (compound **17**, Scheme 4). The SKLVFFA intermediate was assembled on a solid support and its N-terminus serine residue was oxidized in the solution phase using sodium periodate. The ligation was performed in the presence of 50% TFA in a mixture of water–acetonitrile to ensure the solubility of both precursors and limit the aggregation of **17**. The removal of Boc protecting groups from the aminooxy moieties of **11** was accompanied by concomitant removal of the Boc group at the N-1 position and by *in situ* ligation of the KLVFFA peptide leading to **18** in 55% yield. The reaction was monitored by RP-HPLC analysis and a total conversion of **11** was obtained after 1 h reaction at 37 °C.

In order to obtain **20**, two copies of the curcumin alkynyl derivative **19**[21] were attached on "azido" DKP **15** using the Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC). We employed the classical CuAAC combination of copper sulfate and sodium ascorbate as a reductive agent. To avoid the precipitation of **19** due to its strong hydrophobicity, we performed the reaction in the presence of DMF as an organic solvent. We also added THPTA[22] as a water-soluble Cu^{1}-stabilizing ligand. Under these conditions, however, the ligation product **20** was obtained in only 9% yield after RP-HPLC purification. The ligation followed by RP-HPLC showed a total conversion rate of **19** after only 1 h and the low yield is due to loss of product during the purification step.

The orthogonally 'clickable' DKP **16** was employed to prepare the mixed KLVFFA/curcumin DKP **21**. Two copies of the KLVFFA sequence **17** were first ligated *via* oxime bond followed by CuAAC ligation of one copy of the curcumin derivative **19**. If the first ligation step of peptides was realized with a satisfactory yield of 47%, the second ligation step gave **21** in only 23% yield after purification, justifying the 10% overall yield obtained. As mentioned above for **20**, the poor solubility of the product after the ligation of the curcumin molecule in the solvents used for RP-HPLC purification could be involved.

***In vitro* inhibition studies of $A\beta_{40}$ fibril formation.** The inhibitory effect on $A\beta_{40}$ fibril formation of DKPs **18**, **20** and **21** was evaluated using thioflavin T (ThT) fluorescence assays. Synthetic fibrils made from $A\beta_{40}$ monomers and ThT, a specific dye of the characteristic cross-β-sheet structure of fibrils, are commonly used *in vitro* for screening inhibitors.[23] The $A\beta_{40}$ peptide (50 μM) and ThT (10 μM) were co-incubated in phosphate buffer at 37 °C with a concentration range of each DKP (1 to 50 μM) and the ThT fluorescence at 485 nm was measured each day during a period of two weeks (see ESI†). Results summarized in Fig. 2 show the relative change of ThT fluorescence (as compared to Aβ with no inhibitor)

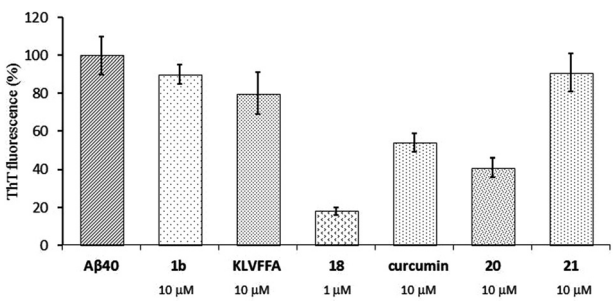


Fig. 2 $A\beta_{40}$ (50 μM) co-incubated with DKPs and controls at the indicated molar concentration. Values are the maximal ThT fluorescence intensity at 485 nm obtained after 7 days compared to that of the control ($A\beta_{40}$ with no inhibitor). Results are the mean ± standard deviation of three experiments.

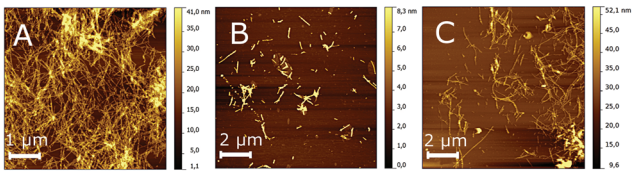


Fig. 3 AFM images (height data) of protofilaments from $A\beta_{40}$ (50 µM) after 8 days of incubation at 37 °C with: (A) no inhibitor, (B) 10 µM of **18**, and (C) 10 µM of **20**.

after 7 days of incubation, from which a maximum of fluorescence was observed. DKP **1b** with no ligand and the ligands alone (KLVFFA peptide and curcumin) were also tested under the same conditions.

At 10 µM concentration, scaffold **1b** and the KLVFFA peptide did not significantly reduce ThT fluorescence signal whereas the DKP **18** bearing two copies of the KLVFFA peptide showed a strong inhibitory activity for a 10-fold lower ratio. Indeed, at 1 µM concentration, the fluorescence signal was reduced by about 80% in the presence of **18**.

This anti-amyloidogenic effect of **18** is consistent with the atomic force microscopy (AFM) observation (Fig. 3). In the control sample of $A\beta_{40}$ with no inhibitor (Fig. 3A), numerous and long fibrils were formed, whereas in the presence of **18** at 10 µM concentration, only a few and shorter fibers were observed (Fig. 3B). The benefit of the dimeric presentation of the KLVFFA motif in DKP **18** is in concordance with previous studies that demonstrated the increased efficiency of KLVFFA multimers against Aβ peptide aggregation.[20,24,25] Especially, the activity of conjugate **18** has a similar activity to that of a cyclodecapeptide scaffold presenting two KLVFFA copies for which we had obtained 90% of inhibition of $A\beta_{40}$ fibril formation for the same Aβ/compound molar ratio.[20]

For the curcumin conjugate **20**, the inhibitory activity was much lower compared to **18** since only 60% of inhibition was observed for a 10-fold higher concentration (Fig. 2). This result was confirmed by the AFM image (Fig. 3C). We had obtained a similar inhibition when two curcumin ligands were ligated onto a cyclodecapeptide scaffold.[20] Moreover, compared to the inhibitory effect of the curcumin ligand alone at the same concentration of 10 µM, **20** presents only a slight benefit. With the mixed DKP **21** at 10 µM, surprisingly, no inhibitory effect on $A\beta_{40}$ fibril formation was shown by the ThT assay (Fig. 2). A 5-fold higher concentration was necessary to obtain 50% of inhibition (see ESI†). In this case, we assume that the assay is affected by the very low solubility of **21** in the aqueous phosphate buffer used.

Through these results, especially through conjugate **18**, the relevance of the DKP scaffold to build efficient *in vitro* Aβ fibril inhibitors is highlighted. This scaffold is of particular interest in this field considering its potentiality to cross the blood–brain barrier.[26] In the dimers of ligands such as **20**, for which the inhibition is quite similar to the ligand alone, the DKP core may be of special interest as cargo to transport the ligands into the brain.

Experimental

Materials and methods

Protected amino acids, especially H-Lys(Boc)-OMe **2b**, PyBOP® and HATU®, were purchased from Calbiochem-Novabiochem and 2-chlorotritylchloride® resin from Advanced ChemTech Europe. Other reagents were obtained from Sigma-Aldrich or Acros Organics.

N-Hydroxysuccinimide esters **8**, **10** and **12** were synthesized as previously described.[17,7a] Curcumin derivative **19** was prepared as reported in the literature from curcumin and propargyl bromide.[21] Imidazole-1-sulfonyl azide hydrochloride **14** was synthesized as reported.[18]

Silica plated aluminum sheets (Silica gel 60 F254) were used for thin-layer chromatography (TLC) and spots were detected by UV (254 nm) or by a solution of 1% ninhydrine in EtOH. For flash chromatography, silica gel 60 (230–400 mesh) was used. RP-HPLC analyses and purifications were performed on Waters equipment consisting of a Waters 600E controller, a Waters 2487 Dual Absorbance Detector, and a Waters In-Line Degasser. UV absorbance was monitored at 214 nm and 250 nm simultaneously. The analytical column (Nucleosil C18, particles size 3 µm, pore size 120 Å, 30 × 4 mm^2) was operated at 1 mL min^{-1} using linear A–B gradients in 20 min run time (solvent A, H_2O containing 0.1% trifluoroacetic acid (TFA); solvent B, CH_3CN containing 9.9% H_2O and 0.1% TFA). The preparative column (Delta-Pak™ 300 Å 15 µm C18 particles, 200 × 25 mm^2) was operated at 22 mL min^{-1} using linear A–B gradients in 30 min run time.

Mass spectra were obtained by electrospray ionization (ESI-MS) on an Esquire 3000 (Bruker) spectrometer in positive mode. The multiply charged data produced by the mass spectrometer on the *m*/*z* scale were converted to the molecular weight. High resolution mass spectra (HRMS) were recorded by the ICOA (Orléans, France) on a Q-Tof MaXis in positive mode.

NMR spectra were recorded on BRUKER Avance 400 and Avance III 500 spectrometers. Chemical shifts are expressed in ppm and calculated taking the solvent peak as an internal reference. Coupling constants are in Hz and signals are described using the usual abbreviations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), *etc*. 1D and 2D NMR techniques such as COSY and ^1H, ^{13}C HSQC have been used for spectral assignments.

In vitro inhibition studies of $A\beta_{40}$ fibril formation were performed using thioflavin T (ThT) assays and atomic force microscopy (AFM) as described previously.[20]

N^{α}-Allyloxycarbonyl-L-lysine methyl ester or H-Lys(Alloc)-OMe (2a). Boc-Lys-OH (3.0 g, 12.20 mmol) was dissolved in 120 mL of water–dioxane (1 : 1) and Et_3N (3.40 mL, 24.50 mmol) was added. The solution is cooled in an ice bath and allylchloroformate (1.95 mL, 18.20 mmol) was added dropwise. After 3 h of stirring at rt, 50 mL of water was added and the pH was adjusted to 2 with 1 M HCl. The mixture was extracted three times with EtOAc and the combined organic phases were washed with water and with brine and dried over

Na_2SO_4. After filtration and concentration of the solvent under vacuum, the residue Boc-Lys(Alloc)-OH was dissolved in 20 mL of MeOH and chlorotrimethylsilane (4 mL, 31.50 mmol) was added dropwise.[27] The solution was stirred overnight at rt and concentrated under vacuum. The residue was triturated with diethyl ether to afford the hydrochloride salt of **2a** (3.10 g, 92%). ESI-MS calcd for $C_{11}H_{20}N_2O_4$ 244.29; found 245.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.01–5.82 (ddt, ^3J = 5.5 Hz, ^3J = 10.5 Hz, ^3J = 17 Hz, 1H, =CHallyl), 5.29 (dd, ^2J = 1.5 Hz, ^3J = 17 Hz, 1H, =CH_2allyl), 5.20 (dd, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.79 (br s, 1H, NHε), 4.55 (d, ^3J = 5.5 Hz, 2H, OCH_2allyl), 3.72 (s, 3H, Me), 3.46 (dd, ^3J = 6.0 and 7.0 Hz, 1H, CHα), 3.18 (dd, ^3J = 7.0 and 13.0 Hz, 2H, CH_2ε), 1.83–1.35 (m, 6H, CH_2β, CH_2γ, CH_2δ). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.02, 157.17, 132.88, 117.71, 65.86, 53.53, 53.27, 50.35, 40.40, 29.69, 28.84, 21.98.

N^{α}(Ns)Lys(Alloc)-OMe (3a). A solution of *o*-nitrobenzosulfonyl chloride (3.20 g, 14.46 mmol) in 4 mL of dioxane was added dropwise at 0 °C to a solution of **2a** (2.9 g, 10.33 mmol) and Et_3N (4.3 mL, 30.10 mmol) in 100 mL of water–dioxane (1 : 1) cooled in an ice bath. The solution was stirred for 30 min at 0 °C and overnight at rt. Water was added and the pH was adjusted at 2 with 1 M HCl. The mixture was extracted three times with EtOAc and the combined organic phases were washed twice with a saturated aqueous solution of $NaHCO_3$, once with brine, and dried over Na_2SO_4. After concentration under vacuum, **3a** was obtained (4.03 g, 91%) as a yellow oil. ESI-MS calcd for $C_{17}H_{23}N_3O_8S$ 429.44; found 430.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–8.01 (m, 1H, HAr), 7.97–7.88 (m, 1H, HAr), 7.80–7.65 (m, 2H, 2 × HAr), 6.13 (d, ^3J = 9.0 Hz, 1H, NHα), 5.99–5.83 (ddt, ^3J = 5.5 Hz, ^3J = 10.5 Hz, ^3J = 17.0 Hz, 1H, =CHallyl), 5.29 (dd, ^2J = 1.5 Hz, ^3J = 17.0 Hz, 1H, =CH_2allyl), 5.20 (dd, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.79 (br s, 1H, NHε), 4.55 (d, ^3J = 5.5 Hz, 2H, OCH_2allyl), 4.16 (td, ^3J = 5.0 Hz, ^3J = 9.0 Hz, 1H, CHα), 3.47 (s, 3H, Me), 3.17–3.15 (m, 2H, CH_2ε), 1.90–1.72 (m, 2H, CH_2β), 1.57–1.37 (m, 4H, CH_2γ, CH_2δ). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.54, 156.49, 147.84, 134.24, 133.79, 132.98, 130.61, 125.79, 117.80, 65.68, 60.54, 56.69, 52.50, 40.57, 32.73, 29.36, 22.25.

N^{α}(Ns)-Lys(Alloc)-OMe (3b). The procedure described above for **3a** was applied from **2b** (2.1 g, 7.08 mmol) to give **3b** (3.09 g, 98% yield) as an oil. ESI-MS calcd for $C_{18}H_{27}N_3O_8S$ 445.48, found 446.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–8.00 (m, 1H, HAr), 7.95–7.85 (m, 1H, HAr), 7.77–7.66 (m, 2H, 2 × HAr), 6.11 (d, ^3J = 7.0 Hz, 1H, NHα), 4.57 (br s, 1H, NHε), 4.20–4.08 (m, 1H, CHα), 3.46 (s, 3H, Me), 3.09–3.07 (m, 2H, CH_2ε), 1.84–172 (m, 2H, CH_2β), 1.53–1.35 (m, 13H, CH_2γ, CH_2δ, *t*Bu).

6-(*N*-*tert*-Butyloxycarbonyl)aminohexan-1-ol (4a). To a solution of 6-aminohexan-1-ol (5 g, 42.70 mmol) in 200 mL of MeOH, di-*tert*-butyl dicarbonate (10.83 g, 49.80 mmol) was added. After 8 h of stirring at rt, the solution was concentrated under vacuum. The residue was dissolved in DCM (250 mL), washed with an aqueous solution brought to pH 3 with 1 M HCl and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was then precipitated in diethyl ether to afford **4a** (7.91 g, 85% yield) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.55 (br s, 1H, NH), 3.71–3.59 (m, 2H, CH_2OH), 3.16–3.09 (m, 2H, *CH*$_2$NH), 1.63–1.32 (m, 17H, 4 × CH_2, *t*Bu).

6-(*N*-Allyloxycarbonyl)aminohexan-1-ol (4b). To a solution of 6-aminohexan-1-ol (3 g, 25.65 mmol) and Et_3N (9 mL, 64.05 mmol) in DCM (120 mL) was added slowly at 0 °C allylchloroformate (4.60 mL, 43.70 mmol). The mixture was then stirred overnight at rt and washed with water, with an aqueous solution brought to pH 2 with 1 M HCl, and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give **4b** (4.68 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (ddt, ^3J = 5.5 Hz, ^3J = 10.5 Hz, ^3J = 17 Hz, 1H, =CHallyl), 5.29 (dd, ^2J = 1.5 Hz, ^3J = 17 Hz, 1H, =CH_2allyl), 5.20 (dd, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.74 (s, 1H, NH), 4.55 (d, ^3J = 5.5 Hz, 2H, OCH_2-allyl), 3.63 (t, ^3J = 6.5 Hz, 2H, CH_2OH), 3.18–3.12 (m, 2H, CH_2NH), 1.61–1.45 (m, 4H, 2 × CH_2), 1.43–1.28 (m, 4H, 2 × CH_2).

N^{α}(*N*-Boc(aminohexyl))-Lys(Alloc)-OMe (5a). To a solution of compound **3a** (3.58 g, 8.33 mmol) in 50 mL anhydrous THF, PPh_3 (3.50 g, 13.33 mmol), **4a** (2.90 g, 13.33 mmol) and DIAD (2.62 mL, 13.33 mmol) were respectively added. The reaction was stirred at rt under argon and was monitored by TLC (EtOAc–cyclohexane, 2 : 1). After completion of the reaction (3 h), the solvent was removed under vacuum and the residue was purified by column chromatography (30 to 40% EtOAc in cyclohexane) to obtain the desired compound from the Mitsunobu reaction in mixture with **4a**. This mixture was dissolved in 80 mL anhydrous DMF and K_2CO_3 (6.72 g, 48.66 mmol) was added. The solution was degassed with argon for 10 min and PhSH (2.49 mL, 24.33 mmol) was introduced dropwise. After 3 h of stirring under argon, water was added and the reaction mixture was extracted three times with diethyl ether. The combined organic phases were dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography (50 to 100% EtOAc in cyclohexane) to provide **5a** (3.32 g, 90%). ESI-MS calcd for $C_{22}H_{41}N_3O_6$ 443.58, found 444.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.91 (ddt, ^3J = 5.5 Hz, ^3J = 10.5 Hz, ^3J = 17 Hz, 1H, =CHallyl), 5.29 (dd, ^2J = 1.5 Hz, ^3J = 17 Hz, 1H, =CH_2allyl), 5.19 (dd, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.85 (br s, 1H, NHε), 4.62–4.50 (m, 3H, OCH_2allyl, NHBoc), 3.73 (s, 3H, Me), 3.26–3.14 (m, 3H, CHα, CH_2ε), 3.13–3.06 (m, 2H, CH_2NHBoc), 2.63–2.53 (m, 1H, CH_2NHα), 2.53–2.41 (m, 1H, CH_2NHα), 1.74–1.6 (m, 2H, CH_2β), 1.60–1.24 (m, 21H, 6 × CH_2, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.72, 156.42, 156.15, 133.17, 117.69, 79.12, 68.11, 65.57, 61.44, 51.92, 48.23, 40.88, 32.96, 30.12, 29.95, 29.81, 28.58, 26.70, 23.06, 21.17.

N^{α}(*N*-Alloc(aminohexyl))-Lys(Boc)-OMe (5b). The procedure described for **5a** was applied for **3b** (2.5 g, 5.60 mmol) and **4b** (1.80 mg, 8.95 mmol) to afford after column chromatography (40 to 50% EtOAc in cyclohexane) the compound from the Mitsunobu reaction in mixture with **4b**. Nosyl removal of the compound from the Mitsunobu reaction was realized on this mixture as described below to give after column chromato-

graphy (50 to 100% EtOAc in cyclohexane) the compound **5b** (2.2 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.98–5.91 (m, 1H, =CHallyl), 5.29 (d, ^3J = 17.0 Hz, 1H, =CH_2allyl), 5.20 (d, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.73 (br s, 1H, NHε), 4.60–4.50 (m, 3H, OCH_2allyl, NHBoc), 3.71 (s, 3H, Me), 3.27–3.13 (m, 3H, CHα, CH_2ε), 3.12–3.04 (m, 2H, CH_2β), 2.62–2.48 (m, 1H, CH_2NHα), 2.48–2.33 (m, 1H, CH_2NHα), 1.65–1.27 (m, 23H, 7 × CH_2, *t*Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.05, 156.29, 155.96, 133.05, 117.55, 79.02, 65.42, 61.45, 51.66, 48.11, 40.97, 40.36, 33.18, 30.05, 29.91, 29.88, 28.44, 26.22, 26.52, 23.08.

N^{α}(Fmoc)-Lys(Alloc)-$N^{(,)\alpha}$(*N*-Boc(aminohexyl))-Lys(Alloc)-OMe (6a). To a mixture of Fmoc-Lys(Alloc)-OH (5.78 g, 12.78 mmol) and HATU (4.86 g, 12.78 mmol) in anhydrous DCM (60 mL) was added DIEA (3.56 mL, 20.44 mmol). The solution was stirred for 30 min under argon, and then a solution of **5a** (2.27 g, 5.11 mmol) in 20 mL anhydrous DCM was added. The solution was stirred under argon and the completion of the reaction was monitored by TLC (EtOAc–cyclohexane, 2 : 1). The mixture was diluted with DCM and was washed with a saturated aqueous solution of $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated under vacuum and the residue purified by column chromatography (40 to 50% EtOAc in cyclohexane) to give **6a** (3.73 g, 83%). RP-HPLC (5 to 100% solvent B) t_R = 18.4 min. ESI-MS calcd for $C_{47}H_{67}N_5O_{11}$ 878.06, found 878.4. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.59 (d, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.40 (t, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.31 (t, ^3J = 7.5 Hz, 2H, 2 × HAr), 5.98–5.81 (m, 2H, 2 × =CHallyl), 5.62 (d, 1H, ^3J = 7.0 Hz, NHα), 5.27 (dd, 2H, ^2J = 1.5 Hz, ^3J = 17.0 Hz, 2 × =CH_2allyl), 5.18 (dd, 2H, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 2 × =CH_2allyl), 4.99 (br s, 2H, 2NHε), 4.69 (br s, 1H, NHBoc), 4.63–4.56 (m, 1H, CHα), 4.53 (d, ^3J = 5.5 Hz, 4H, 2 × OCH_2-allyl), 4.47–4.30 (m, 3H, CHα, CH_2Fmoc), 4.21 (t, ^3J = 7.0 Hz, 1H, CHFmoc), 3.69 (s, 3H, Me), 3.46–3.28 (m, 2H, CH_2N), 3.26–3.01 (m, 6H, 2 × CH_2ε, CH_2NHBoc), 2.14–2.02 (m, 1H, CH_2N), 1.86–1.30 (m, 29H, 10 × CH_2, *t*Bu).

N^{α}(Fmoc)-Lys(Boc)-$N^{(,)\alpha}$(*N*-Alloc(aminohexyl))-Lys(Boc)-OMe (6b). The procedure described for **6a** was applied to Fmoc-Lys (Boc)-OH (4.86 g, 10.36 mmol) and **5b** (1.84 g, 4.14 mmol) to give **6b** (3.5 g, 95%) after column chromatography (50 to 60% EtOAc in cyclohexane). RP-HPLC (5 to 100% solvent B) t_R = 18.9 min. ESI-MS calcd for $C_{48}H_{71}N_5O_{11}$ 894.11, found 894.6. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.78 (d, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.62 (d, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.42 (t, ^3J = 7.5 Hz, 2H, 2 × HAr), 7.33 (t, ^3J = 7.5 Hz, 2H, 2 × HAr), 6.03–5.81 (m, 1H, =CHallyl), 5.66 (br s, 1H, NHα), 5.31–5.22 (m, 1H, =CH_2allyl), 5.18 (d, ^3J = 10.5 Hz, 1H, =CH_2allyl), 5.05 (br s, 1H, NHAlloc), 4.81 (s, 1H, NHε), 4.76 (s, 1H, NHε), 4.69–4.58 (m, 1H, CHα), 4.57–4.50 (m, 2H, OCH_2allyl), 4.49–4.31 (m, 3H, CHα, CH_2Fmoc), 4.24 (t, ^3J = 7 Hz, 1H, CHFmoc), 3.72 (s, 3H, Me), 3.49–3.32 (m, 1H, CH_2N), 3.27–3.01 (m, 6H, 2 × CH_2ε, CH_2NHAlloc), 2.15–2.00 (m, 1H, CH_2N), 1.91–1.31 (m, 38H, 10 × CH_2, 2 × *t*Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.36, 156.37, 156.08, 143.20, 141.33, 133.10, 127.73, 127.07, 125.18, 120.00, 117.46, 79.09, 67.07, 65.37, 53.42, 52.29, 51.00, 47.18, 40.74, 40.34, 33.33, 29.84, 28.69, 28.46, 26.32, 26.09, 23.84, 22.32.

c[N^{ε}-Allyloxycarbonyl-L-lysinyl-$N^{(,)\alpha}$((*N*-*tert*-butyloxycarbonyl)-aminohexyl)-N^{ε}-allyloxycarbonyl-L-lysinyl] or DKP (1a). Compound **6a** (4.0 g, 4.56 mmol) was dissolved in 25mL of a piperidine–DCM solution, 1 : 4. The mixture was stirred for 2 h at rt. After addition of DCM, the organic layer was washed twice with 10% citric acid solution, once with water and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified on column chromatography (2 to 10% MeOH in DCM) to afford compound **1a** (2.47 g, 87%). RP-HPLC (5 to 100% solvent B) t_R = 13.6 min. ESI-MS calcd for $C_{31}H_{53}N_5O_8$ 623.78, found 624.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 (br s, 1H, NH_{lactam}), 5.99–5.83 (m, 2H, 2 × =CHallyl), 5.29 (dd, ^2J = 1.5 Hz, ^3J = 17 Hz, 2H, 2 × =CH_2allyl), 5.20 (dd, ^2J = 1.5 Hz, ^3J = 10.5 Hz, 2H, 2 × =CH_2allyl), 5.00 (s, 2H, 2 × NHε), 4.60–4.50 (m, 5H, 2 × OCH_2allyl, NHBoc), 3.95–3.86 (m, 2H, 2 × CHα), 3.86–3.76 (m, 1H, CH_2N), 3.25–3.15 (m, 4H, 2 × CH_2ε), 3.14–3.00 (m, 2H, CH_2NHBoc), 2.90–2.79 (m, 1H, CH_2N), 2.03–1.90 (m, 2H, CH_2β), 1.68–1.87 (m, 2H, CH_2β), 1.58–1.27 (m, 25H, 8 × CH_2, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.49, 166.20, 156.43, 155.99, 133.01, 117.34, 78.84, 65.30, 59.34, 55.33, 53.43, 44.72, 40.58, 35.41, 32.65, 29.80, 29.59, 29.16, 28.35, 26.81, 26.41, 26.29, 22.70, 22.39.

c[N^{ε}-*tert*-Butyloxycarbonyl-L-lysinyl-$N^{(,)\alpha}$((*N*-allyloxycarbonyl)-aminohexyl)-N^{ε}-*tert*-butylcarbonyl-L-lysinyl] or DKP (1b). The same procedure as described above for **1a** was applied from **6b** (2.5 g, 2.80 mmol) to give **1b** (1.49 g, 83%) after purification by column chromatography (2 to 5% MeOH in DCM). RP-HPLC (5 to 100% solvent B) t_R = 14.5 min. ESI-MS calcd for $C_{32}H_{57}N_5O_8$ 639.82, found 640.5. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.55 (s, 1H, NH_{lactam}), 5.96–5.87 (m, 1H, =CHallyl), 5.30 (dd, ^2J = 1.5 Hz, ^3J = 17.2 Hz, 1H, =CH_2allyl), 5.20 (dd, ^3J = 10.5 Hz, 1H, =CH_2allyl), 4.81 (s, 1H, NH), 4.75–4.60 (br s, 2H, 2 × NH), 4.55 (d, ^3J = 4.9 Hz, 2H, OCH_2allyl), 3.93–3.74 (m, 3H, 2 × CHα, CH_2N), 3.20–3.05 (m, 6H, 2 × CH_2ε, CH_2NHAlloc), 2.89–2.81 (m, 1H, CH_2N), 2.04–1.26 (m, 38H, 10 × CH_2, 2 × *t*Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.96, 166.10, 156.42, 156.20, 133.17, 117.68, 79.35, 79.27, 65.54, 59.56, 55.87, 44.81, 40.95, 40.23, 35.75, 32.86, 29.91, 28.56, 26.95, 26.50, 26.37, 23.11, 22.66.

c[L-Lysinyl-$N^{(,)\alpha}$((*N*-*tert*-butyloxycarbonyl)aminohexyl)-L-lysinyl] or DKP (7a). To a solution of **1a** (2.12 g, 3.40 mmol) in 120 mL of anhydrous DCM under argon, $PhSiH_3$ (8.4 mL, 68 mmol) followed by $Pd(PPh_3)_4$ (79 mg, 68 μmol) were added. The mixture was stirred for 2 h under an inert atmosphere, and then 10 mL of MeOH was added. The solvent was removed under vacuum and the crude was purified by column chromatography (2 to 20% MeOH in DCM in the presence of 5% Et_3N) to give **7a** (1. 38 g, 89%) as a white powder. RP-HPLC (5 to 100% solvent B) t_R = 9.3 min. ESI-MS calcd for $C_{23}H_{45}N_5O_4$ 455.64, found 456.4. RMN ^{1}H (400 MHz, DMSO-d6): δ 8.30 (s, 1H, NH_{lactam}), 6.74 (s, 1H, NHBoc), 3.83–3.80 (m, 1H, CHα), 3.76–3.72 (m, 1H, CHα), 3.65–3.57 (m, 1H, CH_2N), 2.91–2.85 (m, 3H, CH_2NHBoc, CH_2N), 2.61–2.54 (m, 4H, 2 × CH_2ε), 1.88–1.47 (m, 4H, 4 × CH_2β), 1.46–1.15 (m, 25H, 8 × CH_2, *t*Bu). RMN ^{1}H (125 MHz, DMSO-d6): δ 166.72, 165.95,

155.57, 77.29, 58.98, 54.46, 43.97, 35.11, 32.10, 31.17, 30.79, 29.38, 28.28, 26.52, 26.04, 25.98, 22.34, 22.15.

c[N^{ε}-*tert*-Butyloxycarbonyl-L-lysinyl-$N^{(,)\alpha}$(*N*-aminohexyl)-N^{ε}-*tert*-butylcarbonyl-L-lysinyl] or DKP (7b). The same procedure described above for **7a** was applied from **1b** (1.2 g, 1.84 mmol) to give **7b** (930 mg, 91%) after purification (5 to 10% MeOH in DCM in the presence of 1% of Et_3N). RP-HPLC (5 to 100% solvent B) t_R = 11.6 min. ESI-MS calcd for $C_{28}H_{53}N_5O_6$ 555.75, found 556.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.61 (s, 1H, NH_{lactam}), 4.73 (s, 1H, NHBoc), 4.68 (s, 1H, NHBoc), 3.98–3.78 (m, 3H, 2 × CHα, CH_2N), 3.21–3.02 (m, 4H, 2 × $CH_2\varepsilon$), 2.91–2.77 (m, 1H, CH_2N), 2.76–2.59 (m, 2H, CH_2NH_2), 1.97–1.22 (m, 38H, 10 × CH_2, 2 × *t*Bu).

DKP (9). Compound **7a** (46 mg, 0.1 mmol) was dissolved in 1 mL of DCM and the pH was adjusted to 9 with DIEA. Compound **8** (79 mg, 0.22 mmol) was added and the mixture was stirred at rt. The reaction was monitored by analytical RP-HPLC. After 3 h of stirring, the mixture was diluted with AcOEt. The organic layer was washed once with 10% citric acid solution, water and brine. The organic layer was dried on Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by column chromatography (2 to 5% MeOH in DCM) to afford the intermediate DKP (79 mg, 84%) in which two Boc-Ser(*t*Bu) are ligated on the lysine residues. RP-HPLC (5 to 100% solvent B) t_R = 16.6 min. ESI-MS calcd for $C_{47}H_{87}N_7O_{12}$ 942.24, found 942.7. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82 (br s, 1H, NH_{lactam}), 6.71–6.68 (m, 2H, 2 × NHε), 5.46 (br s, 2H, 2 × $NH\alpha_{serine}$), 4.62 (br s, 1H, NHBoc), 4.18–4.11 (br s, 2H, 2 × $CH\alpha_{serine}$), 3.91–3.85 (m, 1H, $CH\alpha_{lysine}$), 3.85–3.79 (m, 2H, $CH\alpha_{lysine}$, CH_2N), 3.77–3.69 (m, 2H, 2 × C$H_2\beta_{serine}$), 3.43–3.33 (m, 2H, 2 × C$H_2\beta_{serine}$), 3.31–3.19 (m, 4H, 2 × $CH_2\varepsilon$), 3.07 (t, ^3J = 6.9 Hz, 2H, CH_2NHBoc), 2.85–2.75 (m, 1H, CH_2N), 2.04–1.88 (m, 2H, 2 × C$H_2\beta_{lysine}$), 1.84–1.62 (m, 2H, 2 × C$H_2\beta_{lysine}$), 1.61–1.24 (m, 43H, 8 × CH_2, 3 × Boc), 1.17 (s, 18H, 2 × CH_2O*t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.82, 170.72, 167.68, 165.97, 156.03, 155.55, 79.99, 79.07, 73.87, 73.84, 61.95, 61.91, 59.39, 55.61, 54.38, 44.76, 40.57, 38.99, 35.52, 32.68, 29.90, 29.19, 29.04, 28.43, 28.34, 27.46, 26.89, 26.48, 26.37, 22.95, 22.60.

This intermediate DKP (79 mg, 0.084 mmol) was dissolved in a mixture of TFA–DCM (1 : 1, 8 mL). The solution was stirred for 2 h at rt and the solvents were removed under vacuum. The residue was taken up in water and lyophilized to give the deprotected DKP, in which serine residues are ligated on the lysine side chains, as TFA salt (53 mg, 73%). RP-HPLC (5 to 40% solvent B) t_R = 4.5 min. ESI-MS calcd for $C_{24}H_{47}N_7O_6$ 529.67, found 530.5. This DKP (27 mg, 0.031 mmol) was dissolved in 4 mL of a CH_3CN–H_2O–TFA (3 : 1.9 : 0.1). $NaIO_4$ (200 mg, 0.93 mmol) was added and the mixture was stirred for 45 min at rt. The solution was purified by preparative RP-HPLC (5 to 40% solvent B) to give **9** (8 mg, 42%) after lyophilization, as TFA salt. RP-HPLC (5 to 40% solvent B) t_R = 5 min. ESI-MS calcd for $C_{22}H_{37}N_5O_6$, $2H_2O$ 503.59, found 504.4.

DKP (11). Compound **7a** (50 mg, 0.11 mmol) was dissolved in 4 mL of DMF and the pH was adjusted to 9 with DIEA. Compound **10** (95 mg, 0.33 mmol) was then added and the solution was stirred at rt for 3 h (completion of the reaction was checked by analytical RP-HPLC). The solvent was removed under reduced pressure and the residue was taken up in DCM, washed with a 5% aqueous $NaHCO_3$ solution, with water and brine. The organic layer was dried on Na_2SO_4, filtered and concentrated under vacuum. The crude was then purified by column chromatography (2 to 6% MeOH in DCM) to afford **11** (67 mg, 76%). RP-HPLC (5 to 100% solvent B) t_R = 13.8 min. ESI-MS calcd for $C_{37}H_{67}N_7O_{12}$ 801.97, found 802.5. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (br s, 1H, ON*H*), 8.33 (br s, 1H, ON*H*), 8.14 (br s, 1H, NHε), 7.99 (br s, 1H, NHε), 7.37 (br s, 1H, NH_{lactam}), 4.66 (br s, 1H, NHBoc), 4.31 and 4.29 (2 s, 2 × 2H, 2 × CH_2ONH), 3.94–3.90 (m, 1H, CHα), 3.87–3.85 (m, 1H, CHα), 3.81–3.75 (m, 1H, CH_2N), 3.28–3.32 (m, 4H, 2 × $CH_2\varepsilon$), 3.08–3.05 (t, ^3J = 7.0 Hz, 2H, CH_2NHBoc), 2.88–2.82 (m, 1H, CH_2N), 1.99–1.93 (m, 2H, 2 × C$H_2\beta$), 1.81–1.68 (m, 2H, 2 × C$H_2\beta$), 1.68–1.27 (m, 43H, 8 × CH_2, 3 × *t*Bu). ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.36, 169.30, 168.13, 166.21, 158.03, 156.21, 82.95, 82.82, 79.19, 76.08, 76.03, 59.47, 55.61, 44.86, 40.52, 38.65, 38.52, 35.33, 32.45, 30.00, 28.74, 28.54, 28.26, 26.98, 26.59, 26.46, 22.82, 22.44.

DKP (13). Compound **7a** (40 mg, 0.088 mmol) was dissolved in 2 mL of DMF and the pH was adjusted to 9 with DIEA. Compound **12** (51 mg, 0.264 mmol) was added and the solution was stirred at rt. The solvent was removed after 3 h under reduced pressure and the residue was purified by column chromatography (0 to 6% MeOH in DCM) to afford the DKP intermediate (46 mg, 85% yield). RP-HPLC (5 to 100% solvent B) t_R = 11.9 min. ESI-MS calcd for $C_{33}H_{53}N_5O_6$ 615.81, found 616.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 1H, ^3J = 2.4 Hz, NH_{lactam}), 6.60 (br s, 1H, NHε), 6.45 (br s, 1H, NHε), 4.66 (br s, 1H, NHBoc), 5.00 (s, 2H, 2 × NHε), 3.92–3.88 (m, 1H, CHα), 3.85–3.83 (m, 1H, CHα), 3.80–3.73 (m, 1H, CH_2N), 3.28–3.24 (m, 4H, 2 × $CH_2\varepsilon$), 3.10–3.02 (m, 2H, CH_2NHBoc), 2.88–2.81 (m, 1H, CH_2N), 2.51–2.47 (m, 4H, 2 × CH_2Csp), 2.40–2.36 (m, 4H, 2 × CH_2CO), 2.04–2.01 (m, 2H, 2 × CHalcyne), 1.98–1.90 (m, 2H, 2 × C$H_2\beta$), 1.81–1.67 (m, 2H, 2 × C$H_2\beta$), 1.65–1.25 (m, 25H, 8 × CH_2, *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.57, 171.52, 168.43, 166.19, 156.20, 83.19, 83.15, 79.21, 69.65, 69.59, 59.49, 55.68, 44.81, 40.65, 39.28, 35.55, 35.38, 35.36, 32.60, 29.99, 29.36, 29.12, 28.55, 26.98, 26.57, 26.46, 22.97, 22.55, 15.15, 15.13. Boc removal of this intermediate (36 mg, 0.058 mmol) was then performed using a mixture of TFA–DCM (1 : 1, 3 mL) for 2 h. After removal of solvents under vacuum and trituration in diethyl ether, **13** (34 mg, 92% yield) was obtained as TFA salt. RP-HPLC (5 to 100% solvent B) t_R = 8.6 min. ESI-MS calcd for $C_{28}H_{45}N_5O_4$ 515.69, found 516.4. ^{1}H NMR (500 MHz, CD_3OD): δ 3.94 (dd, ^3J = 3.8 and 5.4 Hz, 1H, CHα), 3.89 (dd, ^3J = 4.7 and 6.5 Hz, 1H, CHα), 3.75–3.69 (m, 1H, CH_2N), 3.25–3.18 (m, 4H, 2 × $CH_2\varepsilon$), 3.08–3.03 (m, 1H, CH_2N), 2.93 (t, ^3J = 7.5 Hz, 2H, CH_2NH_2), 2.48–2.45 (m, 4H, 2 × CH_2Csp), 2.40–2.36 (m, 4H, 2 × CH_2CO), 2.99 (t, ^3J = 2.4 Hz, 2H, 2 × CHalcyne), 2.03–1.39 (m, 20H, 10 × CH_2). ^{13}C NMR (125 MHz, CD_3OD): δ 173.97, 173.93, 169.62, 168.72, 83.57, 83.54, 70.48, 70.41, 61.11, 56.54, 46.05, 40.59, 40.04, 39.83,

36.63, 36.09, 36.08, 33.51, 29.99, 29.93, 28.37, 27.74, 27.24, 26.94, 24.07, 23.59, 15.82, 15.80.

DKP (15). Compound **7a** (73 mg, 0.16 mmol) was dissolved in 4 mL of DCM, and K_2CO_3 (156 mg, 1.14 mmol) dissolved in 2 mL of water, followed by $ZnCl_2$ (4.4 mg, 0.032 mmol), was added. Then compound **14**[18] (100 mg, 0.48 mmol) dissolved in 4 mL of MeOH was added slowly. The mixture was stirred for 2 h at rt. The solvents were removed under vacuum and the residue was taken up in H_2O. The pH was adjusted to 2 with 1 M HCl and the aqueous phase was extracted three times with DCM. The organic layers were pooled, washed with water, brine, and dried over Na_2SO_4. After filtration and concentration under vacuum, the crude material was purified by column chromatography (0 to 5% MeOH in DCM) to afford the DKP intermediate (64 mg, 78% yield). RP-HPLC (5 to 100% solvent B) t_R = 14.6 min. ESI-MS calcd for $C_{23}H_{41}N_9O_4$ 507.63, found 508.3. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24 (d, ^3J = 2.5 Hz, 1H, NH_{lactam}), 4.56 (br s, 1H, NHBoc), 3.94–3.90 (m, 1H, CHα), 3.89–3.86 (m, 1H, CHα), 3.85–3.79 (m, 1H, CH_2N), 3.31–3.27 (m, 4H, 2 × $CH_2\varepsilon$), 3.09–3.06 (m, 2H, CH_2NHBoc), 2.86–2.79 (m, 1H, CH_2N), 2.03–1.93 (m, 2H, 2 × C$H_2\beta$), 1.84–1.44 (m, 14H, 7 × CH_2), 1.42 (s, 9H, *t*Bu), 1.36–1.24 (m, 4H, 2 × $CH_2\gamma$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.06, 165.94, 156.17, 79.25, 59.45, 55.66, 51.25, 51.22, 44.90, 40.66, 35.62, 32.64, 30.04, 28.72, 28.54, 28.46, 26.99, 26.61, 26.48, 23.11, 22.70. This intermediate (50 mg, 0.098 mmol) was dissolved in a TFA–DCM (1 : 1, 4 mL) mixture. After 2 h of stirring at rt, the solution was concentrated under reduced pressure and the crude was purified by preparative HLPC (5 to 80% solvent B in 20 min run time) to afford **15** (41 mg, 0.078 mmol), as TFA salt, in 80% yield. RP-HPLC (5 to 100% solvent B) t_R = 11.9 min. ESI-MS calcd for $C_{18}H_{33}N_9O_2$ 407.51, found 408.4. ^{1}H NMR (500 MHz, CD_3OD): δ 3.98 (dd, ^3J = 3.8 and 7.1 Hz, 1H, CHα), 3.93 (dd, ^3J = 4.9 and 7.9 Hz, 1H, CHα), 3.76–3.70 (m, 1H, CH_2N), 3.36–3.33 (m, 4H, 2 × $CH_2\varepsilon$), 3.05–3.10 (m, 1H, CH_2N), 2.93 (t, ^3J = 7.6 Hz, 2H, CH_2NH_2), 2.06–1.99 (m, 1H, 1 × C$H_2\beta$), 1.94–1.84 (m, 2H, 2 × C$H_2\beta$), 1.82–1.75 (m, 1H, 1 × C$H_2\beta$), 1.73–1.35 (m, 16H, 8 × CH_2). ^{13}C NMR (125 MHz, CD_3OD): δ 169.56, 168.69, 61.09, 56.48, 52.23, 52.18, 46.07, 40.60, 36.35, 33.25, 29.57, 29.47, 28.41, 27.76, 27.28, 26.97, 23.97, 23.69.

DKP (16). The procedure described above for the synthesis of **15** was applied from **7b** (120 mg, 0.216 mmol) to afford the intermediate bearing one azido function and two free lysine residues (112 mg, 0.184 mmol) in 85% (TFA salt, 2 steps). RP-HPLC (5 to 100% solvent B) t_R = 8.16 min. ESI-MS calcd for $C_{18}H_{35}N_7O_2$ 381.52, found 382.4. This intermediate (110 mg, 0.181 mmol) was dissolved in 4 mL of DMF and the pH was adjusted to 9 with DIEA. Compound **10** (121 mg, 0.42 mmol) was added and the solution was stirred at rt. The completion of the reaction was checked by analytical RP-HPLC and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed once with a 10% citric acid aqueous solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (0 to 5% MeOH in DCM) to afford **16** (88 mg, 0.121 mmol) in 57% yield. RP-HPLC (5 to 100% solvent B) t_R = 14.2 min. ESI-MS calcd for $C_{32}H_{57}N_9O_{10}$ 727.85, found 728.5. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (br s, 1H, NH), 8.19 (br s, 2H, 2 × NHε), 8.00 (br s, 1H, NH), 7.29 (br s, 1H, NH), 4.32 and 4.31 (2d, 2 × 2H, 2 × CH_2ONH), 3.95–3.79 (m, 3H, 2 × CHα, CH_2N), 3.88–3.75 (m, 1H, CH_2N), 3.33–3.30 (m, 4H, 2 × $CH_2\varepsilon$), 3.25 (t, 2H, ^3J = 6.8 Hz, CH_2N_3), 2.88–2.81 (m, 1H, CH_2N), 2.05–1.93 (m, 2H, 2 × C$H_2\beta$), 1.85–1.68 (m, 2H, 2 × C$H_2\beta$), 1.65–1.23 (m, 34H, 8 × CH_2, 2 × *t*Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.46, 168.09, 166.16, 158.05, 83.10, 82.97, 76.15, 76.10, 59.48, 55.68, 51.45, 44.74, 38.69, 38.51, 35.30, 32.28, 28.84, 28.76, 28.28, 26.98, 26.55, 26.51, 22.85, 22.29.

Peptide (17). The protected S(*t*Bu)K(Boc)LVFFA was automatically assembled on 2-chlorotritylchloride resin® (200 mg, 0.6 mmol g^{-1}) using Fmoc/*t*Bu strategy on a Syro II synthesizer using PyBOP as a coupling agent. The peptide was deprotected and released from the resin by treatment for 1 h with 5 mL of a TFA–TIS–H_2O (95/2.5/2.5) solution. The residue obtained after evaporation was precipitated in DCM–Et_2O and the crude material was purified by preparative RP-HPLC (5 to 100% B) to afford the SKLVFFA peptide (50 mg, 0.048 mmol) in 40% yield (TFA salt). RP-HPLC (5 to 100% solvent B) t_R = 10.3 min. ESI-MS calcd for $C_{41}H_{62}N_8O_9$ 810.98, found 811.6. The SKLVFFA peptide (45 mg, 43.3 μmol) was dissolved in 800 μL of CH_3CN and water containing 0.1% TFA (1.2 mL), then $NaIO_4$ (98 mg, 45 μmol) was added. The solution was stirred for 30 min at rt. The reaction mixture was directly purified by preparative RP-HPLC (20 to 70% solvent B) to give **17** (31 mg, 34.0 μmol) in 79% yield (TFA salt). RP-HPLC (5 to 60% solvent B) t_R = 14.2 min. HRMS (ESI) calcd for $C_{40}H_{57}N_7O_9$, H_2O $[M + H]^+$ 798.4396, found 798.4392.

DKP (18). To a solution of DKP **11** (5.4 mg, 7.3 μmol) in 1.3 mL of TFA–H_2O–CH_3CN (5 : 3 : 2), peptide **17** (14 mg, 15.6 μmol) was added and the mixture was heated for 1 h at 37 °C. The mixture was injected in preparative RP-HLPC (20 to 80% solvent B) to give **18** (9.0 mg, 4.0 μmol) in 55% yield, as TFA salt. RP-HPLC (20 to 80% B) t_R = 12.1 min. HRMS (ESI) calcd for $C_{102}H_{153}N_{21}O_{22}$ $[M + 2H]^{2+}$ 1013.0822, found 1013.0831.

DKP (20). DKP **15** (3.4 mg, 6.5 μmol) and curcumin **19** (6.4 mg, 16 μmol) were dissolved in 400 μL of DMF and the solution was degassed with argon (solution 1). To a degassed $CuSO_4{\cdot}5H_2O$ (3.2 mg, 13.0 μmol) solution in water (100 μL), THPTA (12.9 mg, 32.5 mmol) was added followed by sodium ascorbate (13.0 mg, 66.0 μmol) previously dissolved in 300 μL of water (solution 2). Solution 2 was added to solution 1 and the mixed solution was degassed and stirred at rt under argon. The reaction was followed by analytical RP-HPLC and the reaction mixture was purified by preparative HLPC (20 to 100% solvent B) to give **20** as TFA salt (0.91 mg, 0.68 μmol) in 10% yield. RP-HPLC (15 to 100% solvent B) t_R = 13.4 min. HRMS (ESI) calcd for $C_{66}H_{77}N_9O_{14}$ $[M + H]^+$ 1220.5663, found 1220.5662.

DKP (21). To a solution of DKP **16** (3.3 mg, 4.5 μmol) in 1 mL of TFA–H_2O–CH_3CN (5 : 3 : 2), the peptide **17** (10 mg, 11 μmol) was added and the mixture was heated at 37 °C. The

reaction was monitored by analytical RP-HPLC and the reaction mixture was purified by preparative HLPC (5 to 100% solvent) to give, as TFA salt, the intermediate in which two peptides are ligated (4.7 mg, 2.1 μmol) in 47% yield. RP-HPLC (5 to 100% solvent B) t_R = 13.6 min. ESI-MS calcd for $C_{102}H_{151}N_{23}O_{22}$ 2051.44, found 2051.4. This intermediate (4.7 mg, 2.1 μmol) and curcumin **19** (1 mg, 2.52 μmol) were 'clicked' using the same procedure as described for the synthesis of **20**, and the DKP **21** (1.2 mg, 0.49 μmol) was obtained after RP-HPLC purification (5 to 100% solvent B) in 23% yield. RP-HPLC (15 to 100% solvent B) t_R = 13.9 min. HRMS (ESI) calcd for $C_{126}H_{173}N_{23}O_{28}$ $[M + 2H]^{2+}$ 1229.1483, found 1229.1504.

Conclusions

We have developed new "ready-to-use" DKP scaffolds for chemoselective ligations. They are prepared *via* an orthogonal protecting group strategy from Boc/Alloc DKP precursors which are efficiently prepared on a gram scale and which can be easily functionalized with 'clickable' functions. In addition, we have proved their relevance for assembling peptides and organic molecules through the synthesis of potential inhibitors of Aβ amyloid aggregation, the DKP core being of particular interest in the Aβ's target domain due to its potentiality to cross the blood–brain barrier. Our current efforts are devoted to expanding the functional diversity of these scaffolds, especially by exploring other chemoselective and orthogonal ligation protocols.

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