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'Clickable' 2,5-diketopiperazines as scaffolds for ligation of biomolecules: use in A β inhibitor's 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 DKPs precursors prepared from L-lysine residues and an aminohexyl arm, are efficiently prepared in gram scale by sequentially using Fukuyama-Mitsunobu alkylation, dipeptide coupling and diketopiperazine ring formation as key steps. These scaffolds, with their glyoxylyl, aminoxy, alkynyl or azido functions, are "ready-to-use" platforms for biomolecular assembly. Their potentiality in this field is proved through the chemoselective ligation of A β -binding motifs, the KLVFFA peptide and the curcumin molecule. The inhibitory effect of these conjugates on A β 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 they 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 is 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 yet 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 conjugation of biomolecules such as peptides.⁸

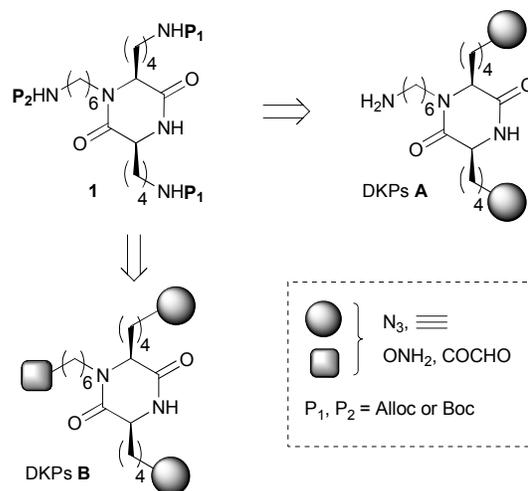


Fig. 1 Chemoselective addressable DKPs targeted in this study.

We chose a cyclo(Lys-Lys) as scaffold to benefit from the chemical addressable amino side chains and then easily introduce 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 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 aminoethyl arm at the *N1*-position of DKPs **A** is to improve the solubility in the aqueous media in whose are usually performed chemoselective ligation of biomolecules. This amino arm may also be used as an additional orthogonally ‘clickable’ site, as shown in the 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* orthogonally protecting group strategy from DKPs **1** as precursors (Fig. 1). We also investigate, through the ligation of well-known A β -binding motifs (the KLVFFA peptide and the curcumin molecule)⁹, their potentiality as scaffolds in A β inhibitor assembly.

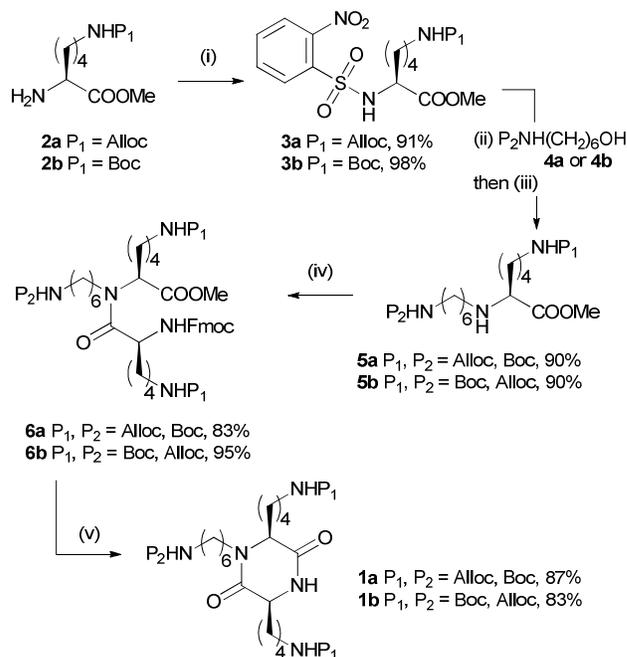
Results and discussion

Synthesis of the DKP precursors

We first prepared the orthogonally protected DKPs **1a** and **1b** ($P_1, P_2 = \text{Boc}$ or Alloc , Scheme 1). The strategy used is a solution-phase synthesis; i.e. *N* α -alkylation of the first lysine partner (**2a** or **2b**), condensation in solution of this *N* α -alkylated *N* ϵ -protected aminoester (**5a** or **5b**) with the second *N* α, ϵ -protected lysine partner, 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 *N1*-position have been reported.^{3a} In this case as in most cases, reductive amination using aldehydes (e.g. glyoxylic aldehyde) is employed for *N* α -alkylation of the first amino acid partner. In our case, a Fukuyama-Mitsunobu reaction^{12,13} is applied for the *N* α -monoalkylation of the first lysine partner using hydroxyl derivatives instead of aldehydes as alkylating agents. The nitrobenzenesulfonyl (Ns) group used both as a protecting and 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 *N* ϵ -Alloc protected lysine methyl ester **2a** was first protected as 2-nitrobenzenesulfonamide with a slight excess of 2-nitrobenzenesulfonyl chloride in the presence of Et₃N. The sulphonamide intermediate **3a**, which was obtained in 91% yield, was then alkylated under the Mitsunobu conditions (DIAD, PPh₃) using the 6-aminohexanol derivative, BocHN(CH₂)₆OH. After removal of the Ns group via aromatic nucleophilic substitution by thiophenol in the presence of K₂CO₃, the *N* α -alkylated lysine aminoester **5a** was obtained in 90% yield from **3a** after chromatography purification. The same sequence was applied to the

commercially available *N* ϵ -Boc protected lysine aminoester **2b** using AllocHN(CH₂)₆OH as alkylating agent to obtain **5b** with a similar yield (88% from **2b**).



(i) Ns-Cl, Et₃N, H₂O/dioxane (1:1), (ii) P₂NH(CH₂)₆OH (**4a** $P_2 = \text{Boc}$, **4b** $P_2 = \text{Alloc}$), PPh₃, DIAD, THF, (iii) PhSH, K₂CO₃, DMF, (iv) Fmoc-Lys(P_1)-OH ($P_1 = \text{Alloc}$ from **5a**, $P_1 = \text{Boc}$ from **5b**), HATU, DIEA, DCM, (v) piperidine/DCM (1:4)

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

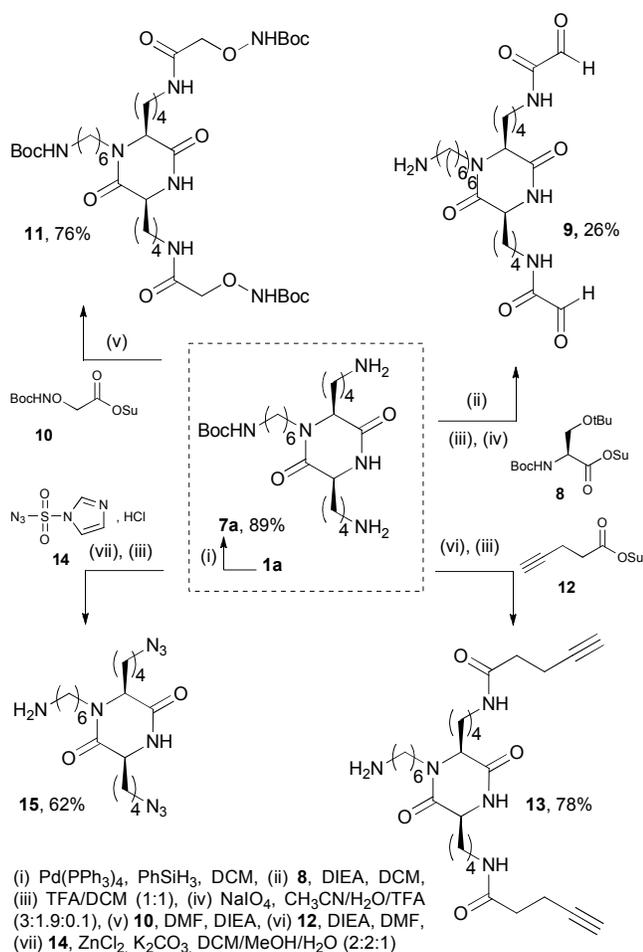
In the literature, *N* α -Boc protected amino acids are often used as second partners. The cyclization is then realized under basic conditions after removal of the *N* α -Boc group under acidic conditions. We chose to use commercially available *N* α -Fmoc protected lysine residues. The coupling of the Fmoc-Lys(Alloc)-OH to the *N* α -alkylated lysine aminoester **5a** was carried out using HATU as 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 dichloromethane¹⁴ 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 to the orthogonally protected DKPs **1a** and **1b** in gram scale in more than 60% overall yield. The protected amino alcohol arms **4a** and **4b** used to introduce the additional functionality in the *N1*-position of the DKP core were easily prepared from the commercial 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 second lysine partner from **5a** or **5b**, DKPs with three orthogonally protecting sites can be also obtained.

Synthesis of the 'clickable' DKPs

The orthogonally protected DKP **1a** was used as common precursor of the 'clickable' DKPs **9**, **11**, **13**, **15** bearing glyoxylyl, aminoxy, 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*-hydroxysuccinimide esters as 'ready-to-use' activated species but the formation *in situ* of the activated species can also be realized.



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

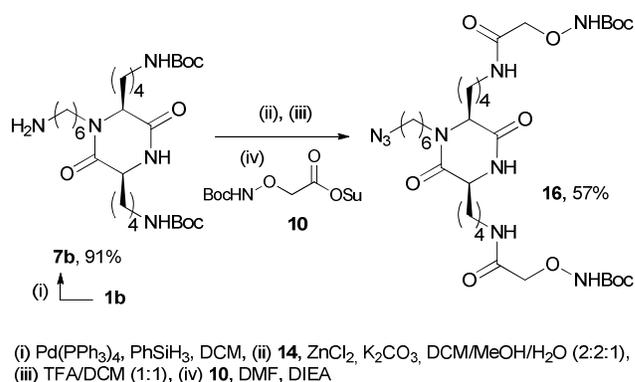
In **9**, the glyoxylyl functions were introduced by coupling the Boc-Ser(*t*Bu)-OSu *N*-hydroxysuccinimide ester **8** on the free lysine residues of **7a**. Boc-Ser(*t*Bu)-OH residue is commonly used as masked glyoxylic acid equivalent in peptide chemistry to attach glyoxylyl groups to the *N*-terminus or to *Nε*-lysine residues of peptides. Indeed, after removal of the Boc and *t*Bu groups, the 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). Losses of product during the last RP-HPLC purification step (only 42% yield) explain this modest yield, while the HPLC analysis of the oxidation reaction mixture shows a total conversion to **9**.

For DKP **11**, the aminoxy functions were incorporated as Boc-protected aminoxyacetyl ester **10**¹⁷ with 76% yield. We preferred for storage keeping the protecting groups due to the high reactivity of the free aminoxy functions towards electrophilic agents. Further oxime ligation being performed in 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 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 aminoxy precursors (compound **16**, Scheme 3).

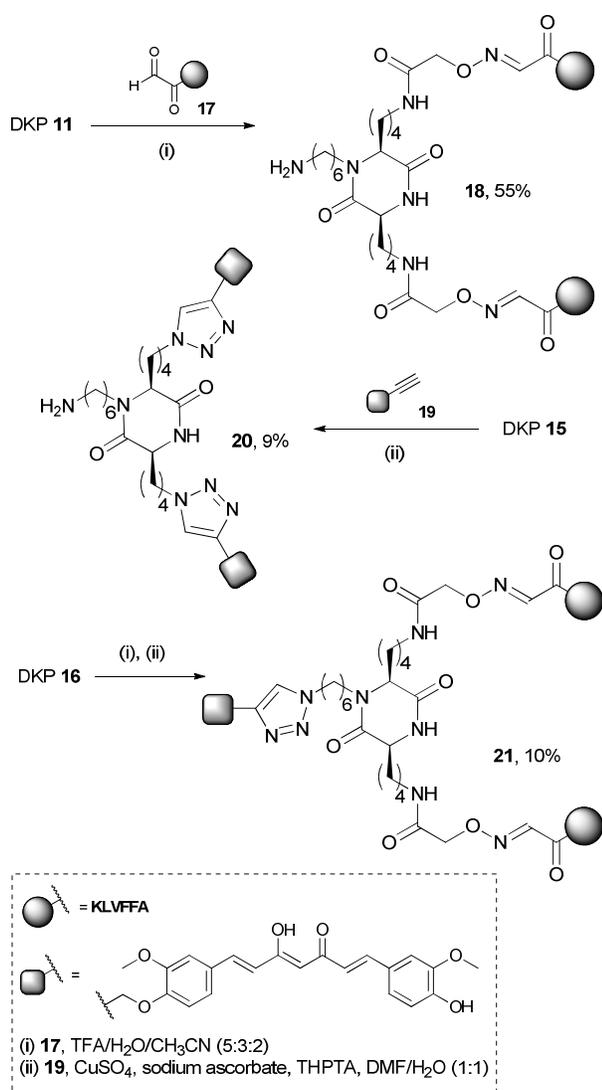


Scheme 3 Synthesis of an orthogonally 'clickable' DKP from **1b**.

Removal of the Alloc group of **1b** was performed, as for **1a**, using the Pd⁰/PhSiH₃¹⁵ procedure affording the DKP **7b** in more than satisfactory yield (91%). The free amino function of **7b** was then converted in azide as mentioned above for **15**. Then, the aminoxy functions were introduced, as Boc-aminoxyacetic 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 from **7a** to obtain the DKP bearing one aminoxy and two azido groups tethered on the lysine side chains.

A β inhibitor assembly

Synthesis. The ‘clickable’ DKPs **11**, **15** and **16** were applied in biomolecular assembly design through the ligation of A β -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 work, we showed that ligation of two copies of KLVFFA or curcumin on a cyclodecapeptide scaffold leads to more potent inhibitors than the A β -binding motifs alone.²⁰ Built to present at least two ligation sites, we were interested in evaluating our DKPs in this field.



Scheme 4 Chemoselective ligation of A β inhibitors.

To assemble the KLVFFA sequence, we chose the oxime bond ligation method from the DKP **11** that presents two masked aminoxy sites. The KLVFFA sequence bearing as complementary function a glyoxylyl group at its *N*-terminus, has been first prepared (compound **17**, Scheme 4). The SKLVFFA intermediate was assembled on solid support and its *N*-terminus serine residue was oxidized in 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 aminoxy moieties of **11** was accompanied by concomitant removal of the Boc group at *N*-1 position and by *in situ* ligation of the KLVFFA peptides 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 reductive agent. To avoid the precipitation of **19** due to its strong hydrophobicity, we performed the reaction in presence of DMF as organic solvent. We also added THPTA²² as water-soluble Cu^I-stabilizing ligand. Under this condition, 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 losses 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** was 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 bad solubility of the product after the ligation of curcumin molecule in the solvents used for RP-HPLC purification could be involved.

In vitro inhibition studies of A β ₄₀ fibril formation. Inhibitory effect on A β ₄₀ fibril formation of DKPs **18**, **20** and **21** was evaluated using Thioflavin T (ThT) fluorescence assay. Synthetic fibrils made from A β 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 A β ₄₀ peptide (50 μ M) and ThT (10 μ M) were co-incubated in phosphate buffer at 37 °C with a concentration range of each DKP (1 to 50 μ M) and the ThT fluorescence at 485 nm was measured each day during a period of two weeks (see ESI). Results summarized in figure 2 show the relative change of ThT fluorescence (as compared to A β with no inhibitor) after 7 days of incubation, from what a maximum of fluorescence was observed. DKP **1b** with no ligand, and the ligands alone (KLVFFA peptide and curcumin) were also tested in the same conditions.

At a 10 μ M concentration, scaffold **1b** and KLVFFA peptide did not significantly reduced ThT fluorescence signal whereas the DKP **18** bearing two copies of the KLVFFA peptides showed a strong inhibitory activity for a 10-fold lower ratio.

Indeed, at a 1 μM concentration, the fluorescence signal was reduced of about 80% in presence of **18**.

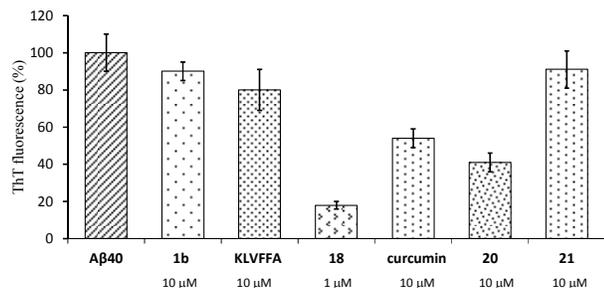


Fig. 2 A β ₄₀ (50 μM) co-incubated with DKPs and controls at the indicated molar concentration. Values are the maximal ThT fluorescence intensity at 485 nm obtained after 7 days compared to that of the control (A β ₄₀ with no inhibitor). Results are the mean \pm standard deviation of three experiments.

This anti-amyloidogenic effect of **18** is consistent with the atomic force microscopy (AFM) observation (Fig. 3). If in the control sample of A β ₄₀ with no inhibitor (Fig. 3A), numerous and long fibrils were formed, in the presence of **18** at a 10 μM concentration, only 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 demonstrating the increased efficiency of KLVFFA multimers against A β peptide aggregation.^{20,24,25} Especially, the activity of conjugate **18** has a similar activity than a cyclodecapeptide scaffold presenting two KLVFFA copies for which we had obtained 90% of inhibition of A β ₄₀ fibril formation for the same A β /compound molar ratio.²⁰

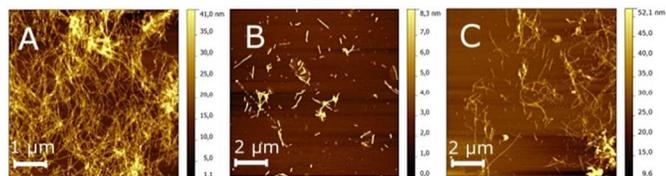


Fig. 3 AFM images (height data) of protofilaments from A β ₄₀ (50 μM) after 8 days of incubation at 37°C with: (A) no inhibitor, (B) 10 μM of **18**, and (C) 10 μM of **20**.

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 β ₄₀ fibril formation was shown by 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 interest of the DKP scaffold to build efficient *in vitro* A β fibril

inhibitors is highlighted. This scaffold is of particular interest in this field considering its potentiality to cross the blood brain barrier.²⁶ In dimers of ligands 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-chlorotriylchloride[®] 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 \times 4 mm²) was operated at 1 mL·min⁻¹ using linear A-B gradients in 20 min run time (solvent A, H₂O containing 0.1% trifluoroacetic acid (TFA); solvent B, CH₃CN containing 9.9% H₂O and 0.1% TFA). The preparative column (Delta-Pak[™] 300 Å 15 μm C18 particles, 200 \times 25 mm²) 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 performed 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)... 1D and 2D NMR techniques such as COSY and ¹H, ¹³C HSQC have been used for spectral assignments.

In vitro inhibition studies of A β 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 Et₃N (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 at 2 with 1M HCl. The mixture was extracted three times with EtOAc and the combined organic phases were washed with water, with brine and dried over Na₂SO₄. 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 C₁₁H₂₀N₂O₄ 244.29; found 245.1. ¹H NMR (400 MHz, CDCl₃): 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, =CH₂allyl), 5.20 (dd, ²J = 1.5 Hz, ³J = 10.5 Hz, 1H, =CH₂allyl), 4.79 (br s, 1H, NHε), 4.55 (d, ³J = 5.5 Hz, 2H, OCH₂allyl), 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, CH₂ε), 1.83–1.35 (m, 6H, CH₂β, CH₂γ, CH₂δ). ¹³C NMR (125 MHz, CDCl₃): 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*-nitrobenzoyl 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 Et₃N (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 1M HCl. The mixture was extracted three times with EtOAc and the combined organic phases were washed twice with a saturated aqueous solution of NaHCO₃, once with brine, and dried over Na₂SO₄. After concentration under *vacuum*, **3a** was obtained (4.03 g, 91%) as a yellow oil. ESI-MS calcd for C₁₇H₂₃N₃O₈S 429.44; found 430.1. ¹H NMR (400 MHz, CDCl₃): δ 8.11–8.01 (m, 1H, HAr), 7.97–7.88 (m, 1H, HAr), 7.80–7.65 (m, 2H, 2xHAr), 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, =CH₂allyl), 5.20 (dd, ²J = 1.5 Hz, ³J = 10.5 Hz, 1H, =CH₂allyl), 4.79 (br s, 1H, NHε), 4.55 (d, ³J = 5.5 Hz, 2H, OCH₂allyl), 4.16 (td, ³J = 5.0 Hz, ³J = 9.0 Hz, 1H, CHα), 3.47 (s, 3H, Me), 3.17–3.15 (m, 2H, CH₂ε), 1.90–1.72 (m, 2H, CH₂β), 1.57–1.37 (m, 4H, CH₂γ, CH₂δ). ¹³C NMR (125 MHz, CDCl₃): 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 C₁₈H₂₇N₃O₈S 445.48, found 446.1. ¹H NMR (400 MHz, CDCl₃): δ 8.10–8.00 (m, 1H, HAr), 7.95–7.85 (m, 1H, HAr), 7.77–7.66 (m, 2H, 2xHAr),

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, CH₂ε), 1.84–1.72 (m, 2H, CH₂β), 1.53–1.35 (m, 13H, CH₂γ, CH₂δ, *t*Bu).

6-(*N*-*tert*-Butyloxycarbonyl)amino)hexan-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 1M HCl and brine. The organic layer was dried over Na₂SO₄, 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. ¹H NMR (400 MHz, CDCl₃): δ 4.55 (br s, 1H, NH), 3.71–3.59 (m, 2H, CH₂OH), 3.16–3.09 (m, 2H, CH₂NH), 1.63–1.32 (m, 17H, 4xCH₂, *t*Bu).

6-(*N*-Allyloxycarbonyl)amino)hexan-1-ol (4b). To a solution of 6-aminohexan-1-ol (3 g, 25.65 mmol) and Et₃N (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 1M HCl, and brine. The organic phase was dried over Na₂SO₄, filtered and concentrated under reduced pressure to give **4b** (4.68 g, 91%). ¹H NMR (400 MHz, CDCl₃): 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, =CH₂allyl), 5.20 (dd, ²J = 1.5 Hz, ³J = 10.5 Hz, 1H, =CH₂allyl), 4.74 (s, 1H, NH), 4.55 (d, ³J = 5.5 Hz, 2H, OCH₂allyl), 3.63 (t, ³J = 6.5 Hz, 2H, CH₂OH), 3.18–3.12 (m, 2H, CH₂NH), 1.61–1.45 (m, 4H, 2xCH₂), 1.43–1.28 (m, 4H, 2xCH₂).

***N*^α(*N*-Boc(amino)hexyl)-Lys(Alloc)-OMe (5a).** To a solution of compound **3a** (3.58 g, 8.33 mmol) in 50 mL anhydrous THF, PPh₃ (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 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 K₂CO₃ (6.72 g, 48.66 mmol) was added. The solution was degassed with argon during 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 Na₂SO₄, 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 C₂₂H₄₁N₃O₆ 443.58, found 444.3. ¹H NMR (400 MHz, CDCl₃): 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, =CH₂allyl), 5.19 (dd, ²J = 1.5 Hz, ³J = 10.5 Hz, 1H, =CH₂allyl), 4.85 (br s,

1H, NH ϵ), 4.62–4.50 (m, 3H, OCH₂allyl, NHBoc), 3.73 (s, 3H, Me), 3.26–3.14 (m, 3H, CH α , CH₂ ϵ), 3.13–3.06 (m, 2H, CH₂NHBoc), 2.63–2.53 (m, 1H, CH₂NH α), 2.53–2.41 (m, 1H, CH₂NH α), 1.74–1.6 (m, 2H, CH₂ β), 1.60–1.24 (m, 21H, 6xCH₂, *t*Bu). ¹³C NMR (100 MHz, CDCl₃): 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 from **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 chromatography (50 to 100% EtOAc in cyclohexane) the compound **5b** (2.2 g, 90% yield). ¹H NMR (400 MHz, CDCl₃): δ 5.98–5.91 (m, 1H, =CHallyl), 5.29 (d, ³*J* = 17.0 Hz, 1H, =CH₂allyl), 5.20 (d, ³*J* = 10.5 Hz, 1H, =CH₂allyl), 4.73 (br s, 1H, NH ϵ), 4.60–4.50 (m, 3H, OCH₂allyl, NHBoc), 3.71 (s, 3H, Me), 3.27–3.13 (m, 3H, CH α , CH₂ ϵ), 3.12–3.04 (m, 2H, CH₂ β), 2.62–2.48 (m, 1H, CH₂NH α), 2.48–2.33 (m, 1H, CH₂NH α), 1.65–1.27 (m, 23H, 7xCH₂, *t*Bu). ¹³C NMR (125 MHz, CDCl₃): 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 ϵ (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 NaHCO₃, water and brine. The organic layer was dried over Na₂SO₄, 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 C₄₇H₆₇N₅O₁₁ 878.06, found 878.4. ¹H NMR (400 MHz, CDCl₃): δ 7.76 (d, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.59 (d, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.40 (t, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.31 (t, ³*J* = 7.5 Hz, 2H, 2xHAr), 5.98–5.81 (m, 2H, 2x=CHallyl), 5.62 (d, 1H, ³*J* = 7.0 Hz, NH α), 5.27 (dd, 2H, ²*J* = 1.5 Hz, ³*J* = 17.0 Hz, 2x=CH₂allyl), 5.18 (dd, 2H, ²*J* = 1.5 Hz, ³*J* = 10.5 Hz, 2x=CH₂allyl), 4.99 (br s, 2H, 2NH ϵ), 4.69 (br s, 1H, NHBoc), 4.63–4.56 (m, 1H, CH α), 4.53 (d, ³*J* = 5.5 Hz, 4H, 2xOCH₂allyl), 4.47–4.30 (m, 3H, CH α , CH₂Fmoc), 4.21 (t, ³*J* = 7.0 Hz, 1H, CHFmoc), 3.69 (s, 3H, Me), 3.46–3.28 (m, 2H, CH₂N), 3.26–3.01 (m, 6H, 2xCH₂ ϵ , CH₂NHBoc), 2.14–2.02 (m, 1H, CH₂N), 1.86–1.30 (m, 29H, 10xCH₂, *t*Bu).

N α (Fmoc)-Lys(Boc)-N ϵ (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 C₄₈H₇₁N₅O₁₁ 894.11, found 894.6. ¹H NMR (400 MHz, CDCl₃): δ 7.78 (d, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.62 (d, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.42 (t, ³*J* = 7.5 Hz, 2H, 2xHAr), 7.33 (t, ³*J* = 7.5 Hz, 2H, 2xHAr), 6.03–5.81 (m, 1H, =CHallyl), 5.66 (br s, 1H, NH α), 5.31–5.22 (m, 1H, =CH₂allyl), 5.18 (d, ³*J* = 10.5 Hz, 1H, =CH₂allyl), 5.05 (br s, 1H, NHAlloc), 4.81 (s, 1H, NH ϵ), 4.76 (s, 1H, NH ϵ), 4.69–4.58 (m, 1H, CH α), 4.57–4.50 (m, 2H, OCH₂allyl), 4.49–4.31 (m, 3H, CH α , CH₂Fmoc), 4.24 (t, ³*J* = 7 Hz, 1H, CHFmoc), 3.72 (s, 3H, Me), 3.49–3.32 (m, 1H, CH₂N), 3.27–3.01 (m, 6H, 2xCH₂ ϵ , CH₂NHAlloc), 2.15–2.00 (m, 1H, CH₂N), 1.91–1.31 (m, 38H, 10xCH₂, 2x*t*Bu). ¹³C NMR (125 MHz, CDCl₃): 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-lysiny]-N ϵ (N-tert-butylloxycarbonyl)aminohexyl)-N ϵ -allyloxycarbonyl-L-lysiny] or DKP (1a). Compound **6a** (4.0 g, 4.56 mmol) was dissolved in 25 mL 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 Na₂SO₄, 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 C₃₁H₅₃N₅O₈ 623.78, found 624.3. ¹H NMR (400 MHz, CDCl₃): δ 6.54 (br s, 1H, NH_{lactame}), 5.99–5.83 (m, 2H, 2x=CHallyl), 5.29 (dd, ²*J* = 1.5 Hz, ³*J* = 17 Hz, 2H, 2x=CH₂allyl), 5.20 (dd, ²*J* = 1.5 Hz, ³*J* = 10.5 Hz, 2H, 2x=CH₂allyl), 5.00 (s, 2H, 2xNH ϵ), 4.60–4.50 (m, 5H, 2xOCH₂allyl, NHBoc), 3.95–3.86 (m, 2H, 2xCH α), 3.86–3.76 (m, 1H, CH₂N), 3.25–3.15 (m, 4H, 2xCH₂ ϵ), 3.14–3.00 (m, 2H, CH₂NHBoc), 2.90–2.79 (m, 1H, CH₂N), 2.03–1.90 (m, 2H, CH₂ β), 1.68–1.87 (m, 2H, CH₂ β), 1.58–1.27 (m, 25H, 8xCH₂, *t*Bu). ¹³C NMR (100 MHz, CDCl₃): 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-butylloxycarbonyl-L-lysiny]-N ϵ (N-allyloxycarbonyl)aminohexyl)-N ϵ -tert-butylcarbonyl-L-lysiny] 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 C₃₂H₅₇N₅O₈ 639.82, found 640.5. ¹H NMR (400 MHz, CDCl₃): δ 6.55 (s, 1H, NH_{lactame}), 5.96–5.87 (m, 1H, =CHallyl), 5.30 (dd, ²*J* = 1.5 Hz, ³*J* = 17.2 Hz, 1H, =CH₂allyl), 5.20 (dd, ³*J* = 10.5 Hz, 1H, =CH₂allyl), 4.81 (s, 1H,

NH), 4.75–4.60 (br s, 2H, 2xNH), 4.55 (d, $^3J = 4.9$ Hz, 2H, OCH₂allyl), 3.93–3.74 (m, 3H, 2xCH α , CH₂N), 3.20–3.05 (m, 6H, 2xCH₂ ϵ , CH₂NHAlloc), 2.89–2.81 (m, 1H, CH₂N), 2.04–1.26 (m, 38H, 10xCH₂, 2xtBu). ¹³C NMR (125 MHz, CDCl₃): 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-lysinyln^(α)((N-tert-butyloxycarbonyl)aminohexyl)-L-lysinyln] or DKP (7a). To a solution of **1a** (2.12 g, 3.40 mmol) in 120 mL of anhydrous DCM under argon, PhSiH₃ (8.4 mL, 68 mmol) followed by Pd(PPh₃)₄ (79 mg, 68 μ mol) were added. The mixture was stirred for 2 h under 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% Et₃N) 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 C₂₃H₄₅N₅O₄ 455.64, found 456.4. RMN ¹H (400 MHz, DMSO-d₆): δ 8.30 (s, 1H, NH_{lactame}), 6.74 (s, 1H, NHBoc), 3.83–3.80 (m, 1H, CH α), 3.76–3.72 (m, 1H, CH α), 3.65–3.57 (m, 1H, CH₂N), 2.91–2.85 (m, 3H, CH₂NHBoc, CH₂N), 2.61–2.54 (m, 4H, 2xCH₂ ϵ), 1.88–1.47 (m, 4H, 4xCH₂ β), 1.46–1.15 (m, 25H, 8xCH₂, tBu). RMN ¹H (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-lysinyln^(α)(N-aminohexyl)-N ^{ϵ} -tert-butylcarbonyl-L-lysinyln] 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 Et₃N). RP-HPLC (5 to 100% solvent B) $t_R = 11.6$ min. ESI-MS calcd for C₂₈H₅₃N₅O₆ 555.75, found 556.3. ¹H NMR (400 MHz, CDCl₃): δ 6.61 (s, 1H, NH_{lactame}), 4.73 (s, 1H, NHBoc), 4.68 (s, 1H, NHBoc), 3.98–3.78 (m, 3H, 2xCH α , CH₂N), 3.21–3.02 (m, 4H, 2xCH₂ ϵ), 2.91–2.77 (m, 1H, CH₂N), 2.76–2.59 (m, 2H, CH₂NH₂), 1.97–1.22 (m, 38H, 10xCH₂, 2xtBu).

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 Na₂SO₄, filtered and concentrated on *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(tBu) are ligated on the lysine residues. RP-HPLC (5 to 100% solvent B) $t_R = 16.6$ min. ESI-MS calcd for C₄₇H₈₇N₇O₁₂ 942.24, found 942.7. ¹H NMR (400 MHz, CDCl₃): δ 6.82 (br s, 1H, NH_{lactame}), 6.71–6.68 (m, 2H, 2xNH ϵ), 5.46 (br s, 2H, 2xNH α _{serine}), 4.62 (br s, 1H, NHBoc), 4.18–4.11 (br s, 2H,

2xCH α _{serine}), 3.91–3.85 (m, 1H, CH α _{lysine}), 3.85–3.79 (m, 2H, CH α _{lysine}, CH₂N), 3.77–3.69 (m, 2H, 2xCH₂ β _{serine}), 3.43–3.33 (m, 2H, 2xCH₂ β _{serine}), 3.31–3.19 (m, 4H, 2xCH₂ ϵ), 3.07 (t, $^3J = 6.9$ Hz, 2H, CH₂NHBoc), 2.85–2.75 (m, 1H, CH₂N), 2.04–1.88 (m, 2H, 2xCH₂ β _{lysine}), 1.84–1.62 (m, 2H, 2xCH₂ β _{lysine}), 1.61–1.24 (m, 43H, 8xCH₂, 3xBoc), 1.17 (s, 18H, 2xCH₂OtBu). ¹³C NMR (100 MHz, CDCl₃): 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 C₂₄H₄₇N₇O₆ 529.67, found 530.5. This DKP (27 mg, 0.031 mmol) was dissolved in 4 mL of a CH₃CN/H₂O/TFA (3:1.9:0.1). NaIO₄ (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 C₂₂H₃₇N₅O₆, 2H₂O 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 NaHCO₃ solution, with water and brine. The organic layer was dried on Na₂SO₄, filtered and concentrated on *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 C₃₇H₆₇N₇O₁₂ 801.97, found 802.5. ¹H NMR (500 MHz, CDCl₃): δ 8.52 (br s, 1H, ONH), 8.33 (br s, 1H, ONH), 8.14 (br s, 1H, NH ϵ), 7.99 (br s, 1H, NH ϵ), 7.37 (br s, 1H, NH_{lactame}), 4.66 (br s, 1H, NHBoc), 4.31 and 4.29 (2s, 2x2H, 2xCH₂ONH), 3.94–3.90 (m, 1H, CH α), 3.87–3.85 (m, 1H, CH α), 3.81–3.75 (m, 1H, CH₂N), 3.28–3.32 (m, 4H, 2xCH₂ ϵ), 3.08–3.05 (t, $^3J = 7.0$ Hz, 2H, CH₂NHBoc), 2.88–2.82 (m, 1H, CH₂N), 1.99–1.93 (m, 2H, 2xCH₂ β), 1.81–1.68 (m, 2H, 2xCH₂ β), 1.68–1.27 (m, 43H, 8xCH₂, 3xtBu). ¹³C NMR (125 MHz, CDCl₃): 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. 1H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, 1H, 3J = 2.4 Hz, $NH_{lactame}$), 6.60 (br s, 1H, $NH\epsilon$), 6.45 (br s, 1H, $NH\epsilon$), 4.66 (br s, 1H, $NHBoc$), 5.00 (s, 2H, $2 \times NH\epsilon$), 3.92–3.88 (m, 1H, $CH\alpha$), 3.85–3.83 (m, 1H, $CH\alpha$), 3.80–3.73 (m, 1H, CH_2N), 3.28–3.24 (m, 4H, $2 \times CH_2\epsilon$), 3.10–3.02 (m, 2H, CH_2NHBoc), 2.88–2.81 (m, 1H, CH_2N), 2.51–2.47 (m, 4H, $2 \times CH_2Csp$), 2.40–2.36 (m, 4H, $2 \times CH_2CO$), 2.04–2.01 (m, 2H, $2 \times CHalcyne$), 1.98–1.90 (m, 2H, $2 \times CH_2\beta$), 1.81–1.67 (m, 2H, $2 \times CH_2\beta$), 1.65–1.25 (m, 25H, $8 \times CH_2$, tBu). ^{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) during 2h. 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. 1H NMR (500 MHz, CD_3OD): δ 3.94 (dd, 3J = 3.8 and 5.4 Hz, 1H, $CH\alpha$), 3.89 (dd, 3J = 4.7 and 6.5 Hz, 1H, $CH\alpha$), 3.75–3.69 (m, 1H, CH_2N), 3.25–3.18 (m, 4H, $2 \times CH_2\epsilon$), 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, $2 \times CH_2Csp$), 2.40–2.36 (m, 4H, $2 \times CH_2CO$), 2.99 (t, 3J = 2.4 Hz, 2H, $2 \times CHalcyne$), 2.03–1.39 (m, 20H, $10 \times CH_2$). ^{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) were 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 1M 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. 1H NMR (400 MHz, $CDCl_3$): δ 7.24 (d, 3J = 2.5 Hz, 1H, $NH_{lactame}$), 4.56 (br s, 1H, $NHBoc$), 3.94–3.90 (m, 1H, $CH\alpha$), 3.89–3.86 (m, 1H, $CH\alpha$), 3.85–3.79 (m, 1H, CH_2N), 3.31–3.27 (m, 4H, $2 \times CH_2\epsilon$), 3.09–3.06 (m, 2H, CH_2NHBoc), 2.86–2.79 (m, 1H, CH_2N), 2.03–1.93 (m, 2H, $2 \times CH_2\beta$), 1.84–1.44 (m, 14H, $7 \times CH_2$), 1.42 (s, 9H, tBu), 1.36–1.24 (m, 4H, $2 \times CH_2\gamma$). ^{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. 1H NMR (500 MHz, CD_3OD): δ 3.98 (dd, 3J = 3.8 and 7.1 Hz, 1H, $CH\alpha$), 3.93 (dd, 3J = 4.9 and 7.9 Hz, 1H, $CH\alpha$), 3.76–3.70 (m, 1H, CH_2N), 3.36–3.33 (m, 4H, $2 \times CH_2\epsilon$), 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, $1 \times CH_2\beta$), 1.94–1.84 (m, 2H, $2 \times CH_2\beta$), 1.82–1.75 (m, 1H, $1 \times CH_2\beta$), 1.73–1.35 (m, 16H, $8 \times CH_2$). ^{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 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. 1H NMR (400 MHz, $CDCl_3$): δ 8.38 (br s, 1H, NH), 8.19 (br s, 2H, $2 \times NH\epsilon$), 8.00 (br s, 1H, NH), 7.29 (br s, 1H, NH), 4.32 and 4.31 (2d, $2 \times 2H$, $2 \times CH_2ONH$), 3.95–3.79 (m, 3H, $2 \times CH\alpha$, CH_2N), 3.88–3.75 (m, 1H, CH_2N), 3.33–3.30 (m, 4H, $2 \times CH_2\epsilon$), 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, $2 \times CH_2\beta$), 1.85–1.68 (m, 2H, $2 \times CH_2\beta$), 1.65–1.23 (m, 34H, $8 \times CH_2$, $2 \times tBu$). ^{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(tBu)K(Boc)LVFFA was automatically assembled on 2-chlorotritylchloride resin[®] (200 mg, 0.6 mmol.g⁻¹) using Fmoc/ tBu strategy on a Syro II synthesizer using PyBOP as coupling agent. The peptide was deprotected and released from the resin by treatment during 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 μ mol) was dissolved in 800 μ L of CH_3CN and water containing 0.1% TFA (1.2 mL), then $NaIO_4$ (98 mg, 45 μ mol) 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 μ mol) 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 μ mol) in 1.3 mL of TFA/ H_2O / CH_3CN (5:3:2), peptide **17** (14 mg, 15.6 μ mol) was added and the mixture was heated 1 h at 37 $^{\circ}C$. The mixture was injected in preparative RP-HPLC (20 to 80% solvent B) to give **18** (9.0 mg, 4.0 μ mol) 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 μ mol) and curcumin **19** (6.4 mg, 16 μ mol) were dissolved in 400 μ L of DMF and the solution was degassed with argon (solution 1). To a degassed $CuSO_4 \cdot 5H_2O$ (3.2 mg, 13.0 μ mol) solution in water (100 μ L), THPTA (12.9 mg, 32.5 mmol) was added followed by sodium ascorbate (13.0 mg, 66.0 μ mol) previously dissolved in 300 μ L of water (solution 2). The solution 2 was added to the 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 HPLC (20 to 100% solvent B) to give **20** as TFA salt (0.91 mg, 0.68 μ mol) 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 μ mol) in 1 mL of TFA/ H_2O / CH_3CN (5:3:2), the peptide **17** (10 mg, 11 μ mol) was added and the mixture was heated at 37 $^{\circ}C$. The reaction was monitored by analytical RP-HPLC and the reaction mixture was purified by preparative HPLC (5 to 100% solvent) to give, as TFA salt, the intermediate in which two peptides are ligated (4.7 mg, 2.1 μ mol) 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 μ mol) and curcumin **19** (1 mg, 2.52 μ mol) were 'clicked' using the same procedure as described for the synthesis of **20**, and the DKP **21** (1.2 mg, 0.49 μ mol) 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* orthogonally protecting group strategy from Boc/Alloc DKP precursors which are efficiently prepared in gram scale and which can be easily functionalized with 'clickable' functions. In addition, we have proved their interest for assembling peptides and organic molecules, through the synthesis of potential inhibitors of A β amyloid aggregation; the DKP core being of particular interest in the A β 's target domain due to its potentiality to cross the blood brain barrier. Our current efforts are devoted to expand the functional diversity of these scaffolds, especially by exploring other chemoselective and orthogonal ligation protocols.

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Notes and references

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- 1 A. D. Borthwick, *Chem. Rev.*, 2012, **112**, 3641.
- 2 M. Marchini, M. Mingozzi, R. Colombo, I. Guzzetti, L. Belvisi, F. Vasile, D. Potenza, U. Piarulli, D. Arosio and C. Gennari, *Chem. Eur. J.*, 2012, **18**, 6195.
- 3 (a) G. Gellerman, E. Hazan, T. Brider, T. Traube, A. Albeck and S. Shatzmiler, *Int. J. Pept. Res. Ther.*, 2008, **14**, 183. (b) G. Gellerman, E. Hazan, M. Kovaliov, A. Albeck and S. Shatzmiler, *Tetrahedron*, 2009, **65**, 1389.
- 4 G. Gellerman, US Pat., 20100210474 A1.
- 5 M. Tullberg, M. Grotli and K. Luthman, *J. Org. Chem.*, 2007, **72**, 195.
- 6 E. V. Dzyuba, L. Kaufmann, N. L. L \ddot{o} w, A. K. Meyer, H. D. F. Winkler, K. Rissanen and C. A. Schalley, *Org. Lett.*, 2011, **13**, 4838. In this paper, the synthesis of a *N,N'*-dipropargyl 2,5-diketopiperazine derived from glycine residues is reported to prepare by 'click chemistry' rotaxanes but this scaffold could be used to ligate biomolecules.
- 7 (a) G. M. Galibert, P. Dumy and D. Boturyn, *Angew. Chem. Int. Ed.*, 2009, **48**, 2576. (b) O. Renaudet, J. Garcia, D. Boturyn, N. Spinelli,

- E. Defrancq, P. Labbé and P. Dumy, *Int. J. Nanotechnology*, 2010, **7**, 738.
- 8 (a) M. Sletten and C. R. Bertozzi, *Angew. Chem. Int. Ed.*, 2009, **48**, 2. (b) J. Kalia and R. T. Raines, *Curr. Org. Chem.*, 2010, **14**, 138.
- 9 (a) M. A. Findeis, G. M. Musso, C. C. Arici-Muendel, H. W. Benjamin, A. M. Hundal, J. J. Lee, J. Chin, M. Kelley, J. Wakefield, N. J. Hayward and S. M. Molineaux, *Biochemistry*, 1999, **38**, 6791. (b) Y. Porat, A. Abramowitz and E. Gazit, *Chem. Biol. Drug. Des.*, 2006, **67**, 27.
- 10 A. S. M. Ressurreição, A. Bordessa, M. Civera, L. Belvisi, C. Gennari and U. Piarulli, *J. Org. Chem.*, 2008, **73**, 652.
- 11 C. J. Dinsmore and D. C. Beshore, *Tetrahedron*, 2002, **58**, 3297.
- 12 (a) T. Fukuyama, C. -K. Jow and M. Cheung, *Tetrahedron Lett.*, 1995, **36**, 6373. (b) T. Kan and T. Fukuyama, *Chem. Commun.*, 2004, 353.
- 13 O. Demmer, I. Dijkgraaf, M. Schottelius, H. -J. Wester and H. Kessler, *Org. Lett.*, 2008, **10**, 2015.
- 14 P. Besada, L. Mamedova, C. J. Thomas, S. Costanzi and K. A. Jacobson, *Org. Biomol. Chem.*, 2005, **3**, 2016.
- 15 M. Dessolin, M. G. Guillerez, N. Thieriet, F. Guibé and A. Loffet, *Tetrahedron Lett.*, 1995, **36**, 5741.
- 16 O. El-Mahdi and O. Melnyk, *Bioconjugate Chem.*, 2013, **24**, 735.
- 17 S. Foillard, M. O. Rasmussen, J. Razkin, D. Boturyn and P. Dumy, *J. Org. Chem.*, 2008, **73**, 983.
- 18 E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797 (Additions and Corrections to this article in *Org. Lett.* 2011, **13**, 2514).
- 19 (a) For a more safe and at large-scale protocol than Goddard-Borger's protocol to prepare imidazole-1-sulfonyl azide see: H. Ye, R. Liu, D. Li, Y. Liu, H. Yuan, W. Guo, L. Zhou, X. Cao, H. Tian, J. Shen and P. G. Wang, *Org. Lett.*, 2013, **15**, 18. (b) For more safe alternatives to the hydrogen chloride salt as the hydrogen sulfate salt see: N. Fischer, E. D. Goddard-Borger, R. Greiner, T. M. Klapötke, B. W. Skelton and J. Stierstorfer, *J. Org. Chem.*, 2012, **77**, 1760.
- 20 M. Ouberaï, P. Dumy, S. Chierici and J. Garcia, *Bioconjugate Chem.*, 2009, **20**, 2123.
- 21 W. Shi, S. Dolai, S. Rizk, A. Hussain, H. Tariq, S. Haverick, W. L'Amoreaux, A. El Idrissi, P. Banerjee and K. Raja, *Org. Lett.*, 2007, **9**, 5461.
- 22 (a) V. Hong, S. I. Presolski, C. Ma and M. G. Finn, *Angew. Chem.*, 2009, **121**, 10063. (b) C. Besanceney-Webler, H. Jiang, T. Zheng, L. Feng, D. Soriano del Amo, W. Wang, L. M. Klivansky, F. L. Marlow, Y. Liu and P. Wu, *Angew. Chem. Int. Ed.*, 2011, **50**, 8051.
- 23 (a) H. LeVine, *Protein Sc.*, 1993, **2**, 404. (b) H. LeVine, *Methods Enzymol.*, 1999, **309**, 274.
- 24 G. Zhang, M. J. Leibowitz, P. J. Sinko and S. Stein, *Bioconjugate Chem.*, 2003, **14**, 86.
- 25 S. M. Chafekar, H. Malda, M. Merckx, E. W. Meijer, D. Viertl, H. A. Lashuel, F. Baas and W. Scheper, *ChemBioChem*, 2007, **8**, 1857.
- 26 M. Teixido, E. Zurita, M. malakoutikhah, T. Tarrago and E. Giralt, *J. Am. Chem. Soc.*, 2007, **129**, 11802.
- 27 (a) B. -C. Chen, A. P. Skoumbourdis, P. Guo, M. S. Bednarz, O. R. Kocy, J. E. Sundeen and G. D. Vite, *J. Org. Chem.*, 1999, **64**, 9294. (b) J. Li and Y. Sha, *Molecules*, 2008, **13**, 1111.