Supplementary Material (ESI) for Chemical Science

Electronic Supplementary Information

Amyloidogenesis highlighted by designed peptides forming supramolecular self-assemblies

M. Ouberai, G. T. Dolphin, P. Dumy, and J. Garcia*

Département de Chimie Moléculaire, UMR-CNRS 5250, ICMG FR 2607,
Université Joseph Fourier, BP53, 38041 Grenoble Cedex 9, France

E-mail: julian.garcia@ujf-grenoble.fr

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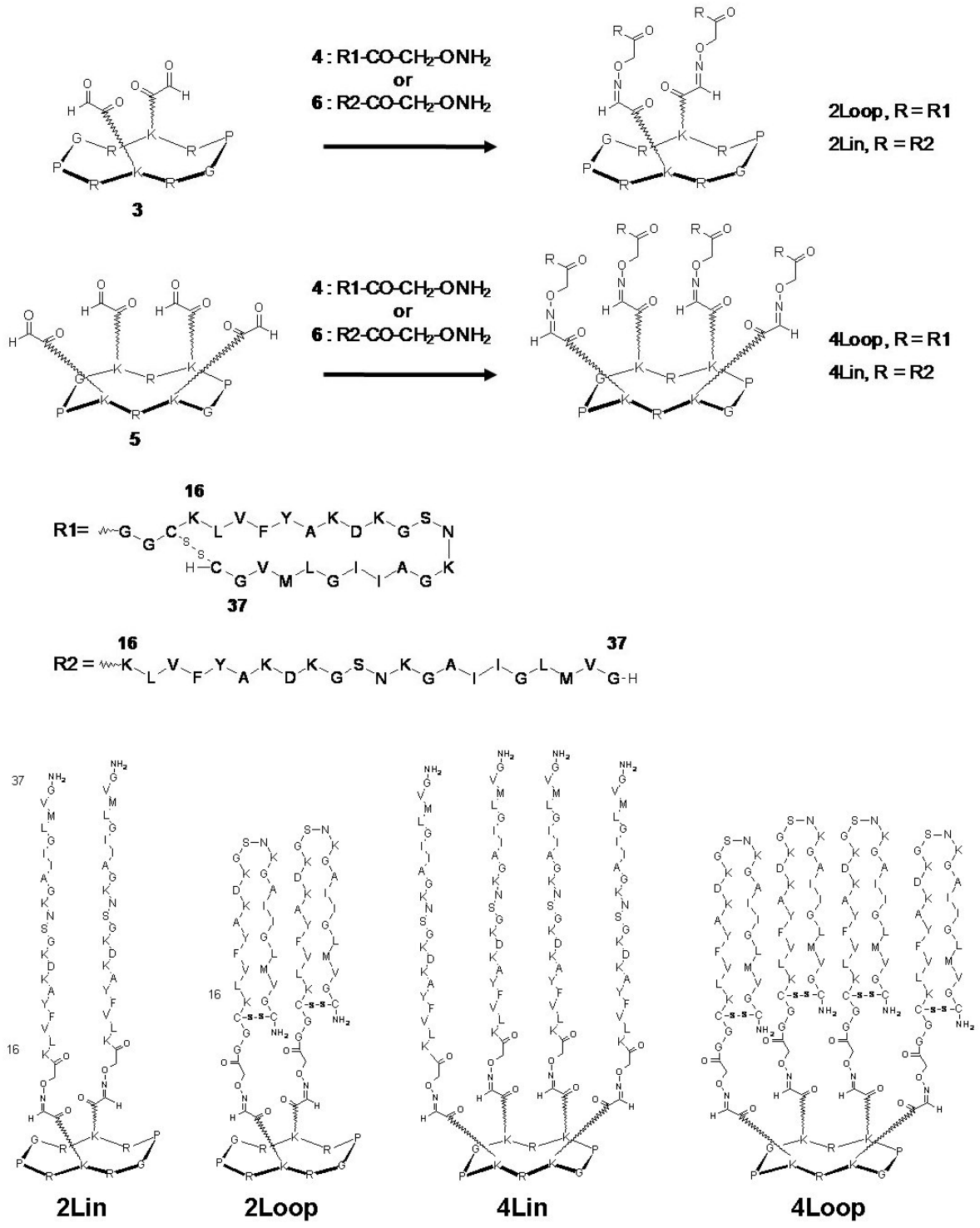


Figure S1. Chemical structures of peptide edifices and corresponding precursors.

Synthesis of cyclic decapeptide 3 (Figure S2). The linear peptide R(Pmc)-K(Dde)-R(Pmc)-P-G-R(Pmc)-K(Dde)-R(Pmc)-P-G was first built up automatically (Advance Chem Tech 348 Ω peptide synthesizer) on Fmoc-Gly-Sasrin® resin (500 mg, 0.69 mmol.g^{-1}) and cyclized (415

mg, $1.59.10^{-4}$ mol) in DMF (0.5 mmol.L^{-1}) under high dilution using PyBOP (1.2 eq.) and DIPEA. The white solid powder obtained after precipitation and washing in diethyl ether was solubilized in a solution of 2% of hydrazine in DMF to remove Dde protecting groups. The cyclopeptidic intermediate **1** (272 mg, $1.21.10^{-4}$ mol) was then obtained after precipitation and washing in diethyl ether (76% yield from the linear peptide). To a solution of compound **1** (272 mg, $1.21.10^{-4}$ mol) in DMF (0.01 mol.L^{-1}), were added Boc-Ser(*t*Bu)-OH (3 eq), PyBOP (3 eq.) and DIPEA. The mixture was stirred for 2 h at r.t. Then Boc, *t*Bu and Pmc protecting groups were removed using a solution of TFA/TIS/H_2O (95:2.5:2.5) (0.01 mol.L^{-1}). After 2 h, the solvent was evaporated and the crude compound **2** (145 mg, $7.08.10^{-5}$ mol) was obtained by precipitation with diethyl ether as white solid powder with 59% yield. HPLC t_R = 4.9 min. ESI-MS calc 1362.8, found 1362.5.

To a solution of compound **2** (5 mg, $2.44.10^{-6}$ mol) in H_2O/CH_3CN (1:1) (0.01 mol.L^{-1}) was added $NaIO_4$ (20 eq.). The reaction was stirred for 20 min at r.t. and immediately purified by RP-HPLC (C18 Nucleosil® column, 5-100% B in 30 min). This procedure was realized 8 times to afford compound **3** (21 mg, $1.19.10^{-5}$ mol) as a white powder with 61% yield. HPLC t_R = 5.0 min. ESI-MS calc 1300.7, found 1300.4.

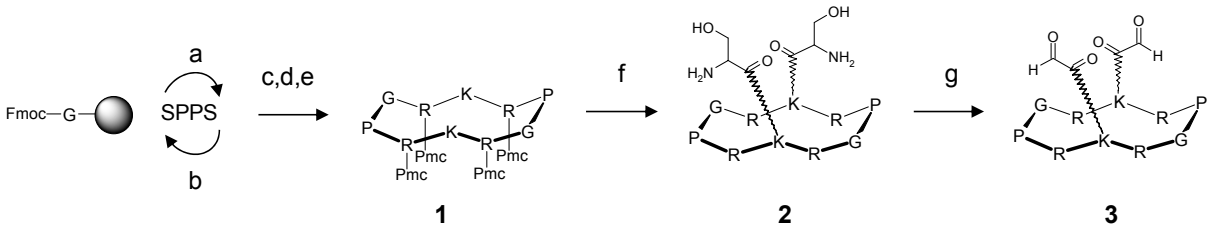
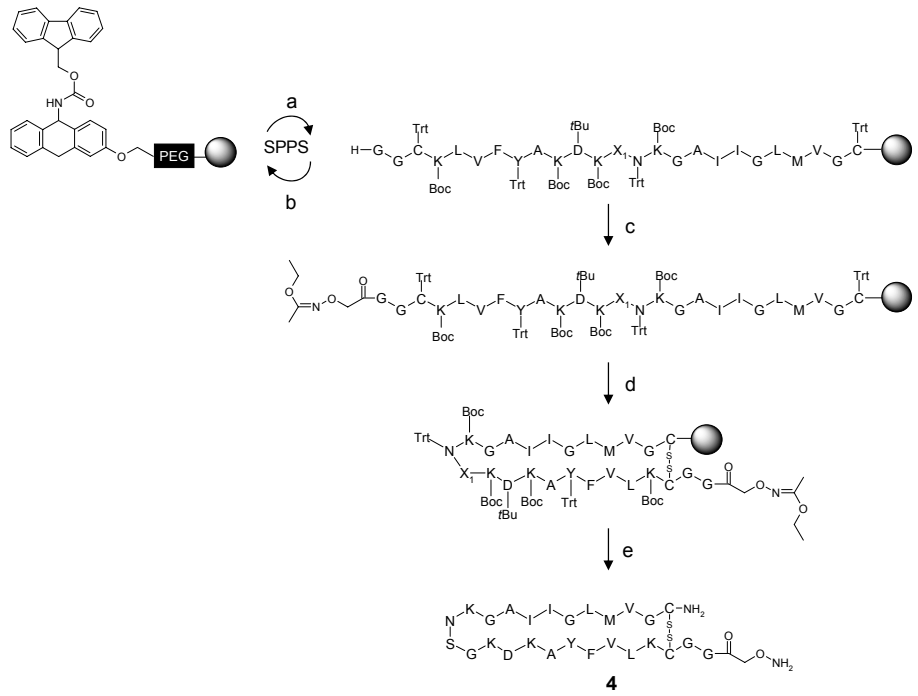


Figure S2. Synthesis of the cyclodecapeptide **3**. (a) Piperidine/DMF (1:4); (b) Fmoc-Xaa-OH (2 eq.), PyBOP (2 eq.), DIPEA (3-4 eq.), DMF; (c) TFA/CH_2Cl_2 (1:99), 10 min (three times); (d) PyBOP (1.2 eq.), DIPEA (3-4 eq.), DMF (0.5 10^{-3} M), 1 h; (e) Hydrazine/DMF (2:98), 2 h, 76% from the linear form of **1**; (f) i) Boc-Ser(*t*Bu)-OH (3 eq.), PyBOP (3 eq.), DIPEA, DMF (10^{-2} M), 2 h; ii) TFA/TIS/H_2O (95:2.5:2.5), 2 h, 59% from **1** (2 steps); (g) $NaIO_4$ (20 eq.), H_2O/CH_3CN (1:1), 61%.

Synthesis of peptide 4 (Figure S3). The GGCA$\beta_{16\text{-}37}Y_{20}K_{22}K_{24}$C peptide sequence was synthesized automatically (Applied Biosystems) by solid phase synthesis on NovaSyn® TG Sieber resin (300 mg, 0.19 mmol.g^{-1}). Peptide on resin ($5.70.10^{-5}$ mol) was then solvated in 10 mL of DMF and the pH was adjusted with DIEPA to pH 8-9. 2-(1-ethoxyethylideneaminooxy)acetic acid (2 eq.), and PyBOP (2 eq.) were added to the resin

solution. The mixture was stirred for 1 h at r.t. Peptide was then solvated in DMF and iodine (20 eq.) was added. The peptide was released from the resin using 10 mL cleavage solution of TFA/H_2O/TIS (95:2.5:2.5). The mixture was stirred for 2 h at r.t then 10 eq. of NH_4I was added and the mixture was stirred for another 30 min. The crude free peptide **4** was obtained as a white powder (142 mg, $4.34.10^{-5}$ mol) and then purified by RP-HPLC (C18 Nucleosil® column, 5-100% B in 30 min) affording pure peptide **4** (15.1 mg, $4.62.10^{-6}$ mol) as a white powder with 8% overall yield from the resin. HPLC t_R = 8.3 min. ESI-MS calc 2698.4, found 2699.1.



X_1 = G-S($\psi^{Me,Me}$pro)

Figure S3. Synthesis of the peptide **4**. (a) Piperidine/NMP (1:4); (b) Fmoc-Xaa-OH (10 eq.), HBTU (10 eq.), DIPEA (20 eq.), NMP; (c) 2-(1-ethoxyethylideneaminooxy)acetic acid (2 eq.), PyBOP (2 eq.), DIPEA (3-4 eq.), DMF; (d) I_2 (20 eq.), DMF; (e) TFA/H_2O/TIS (95:2.5:2.5), NH_4I (10 eq.), 8% from the resin.

The cyclic decapeptide **5** and the peptide **6** (Figure S1) were synthesized as previously described.[1] Synthetic $A\beta_{1-40}$ was prepared as previously described.[2]

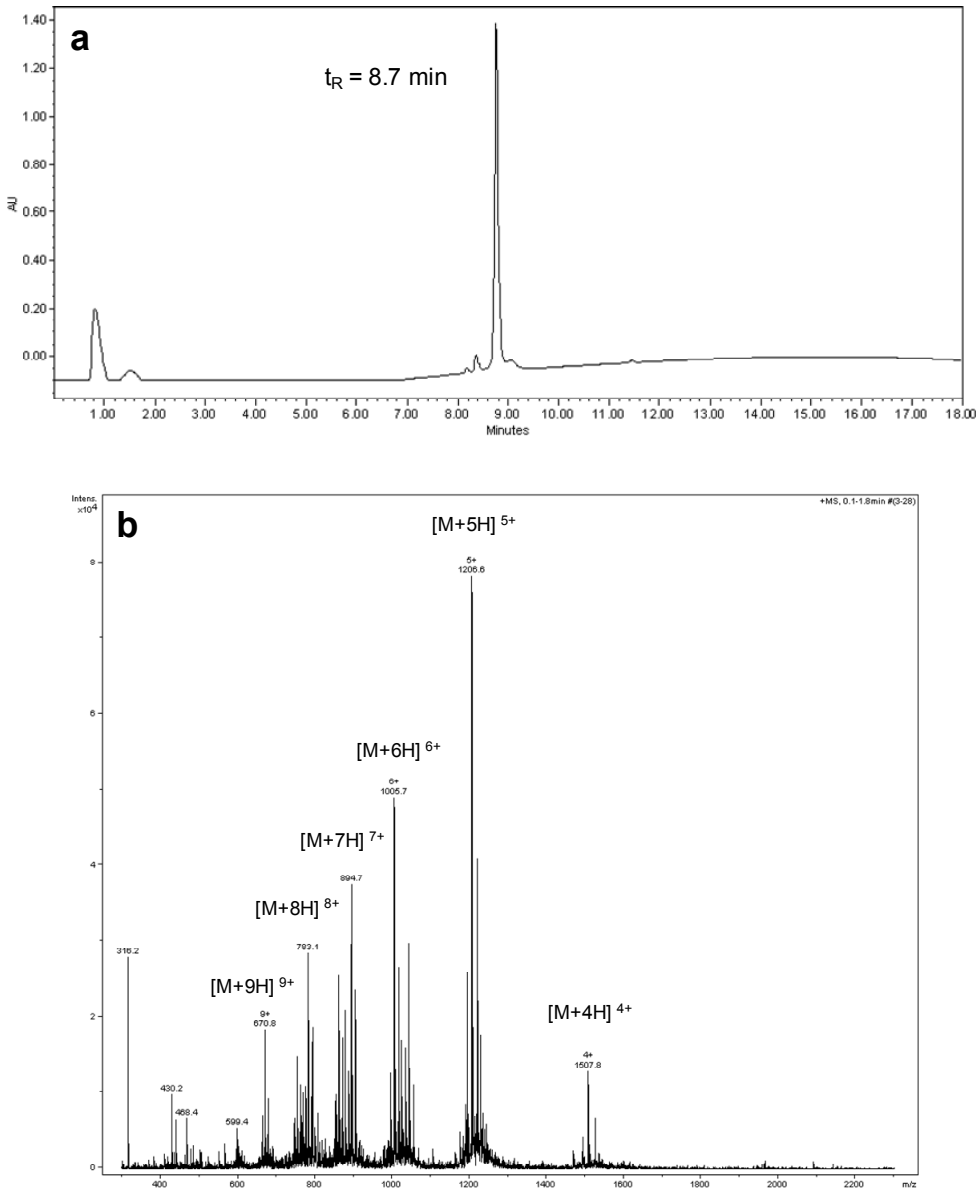


Figure S4. Characterization of compound **2Lin** by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).

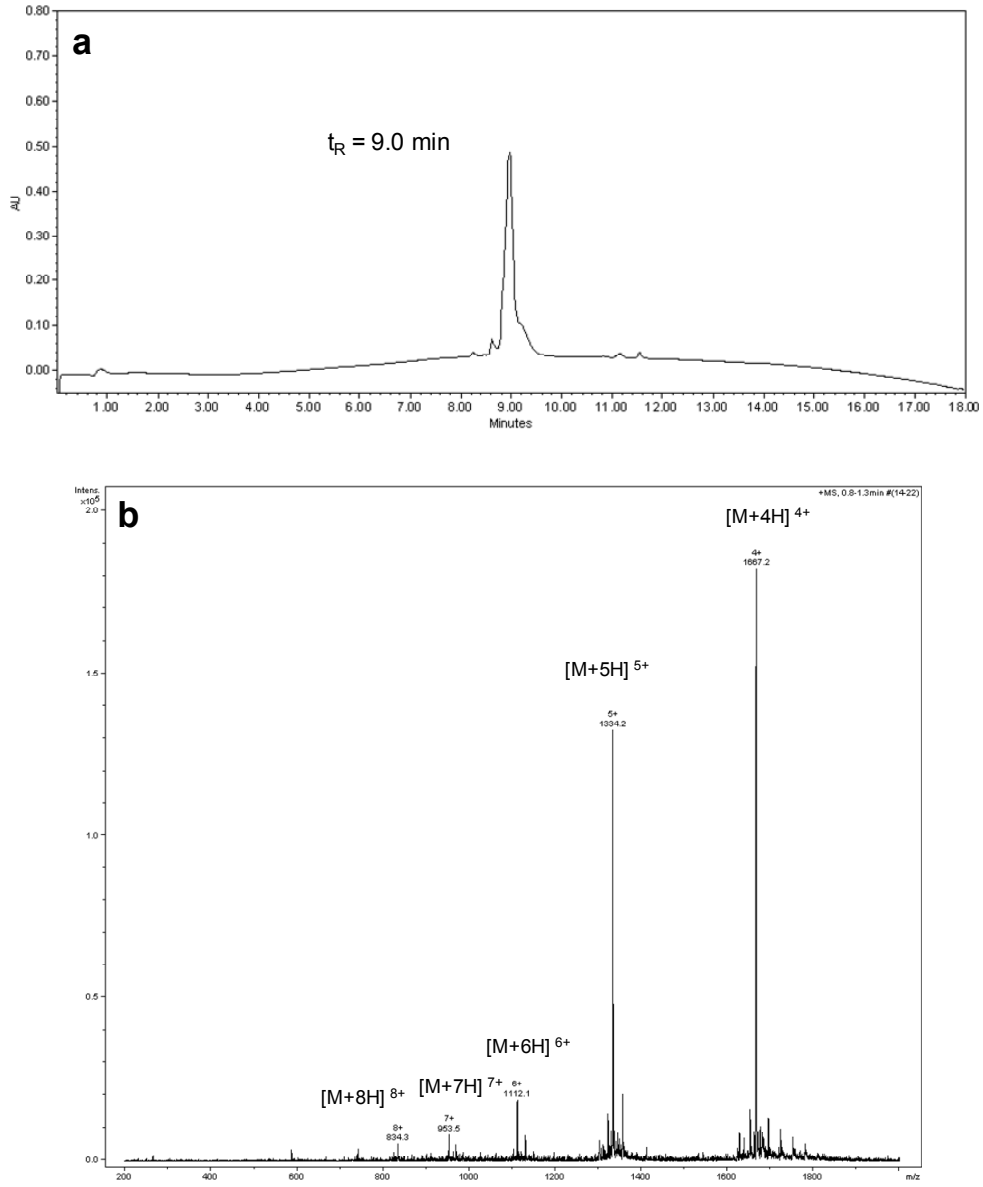


Figure S5. Characterization of compound **2Loop** by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).

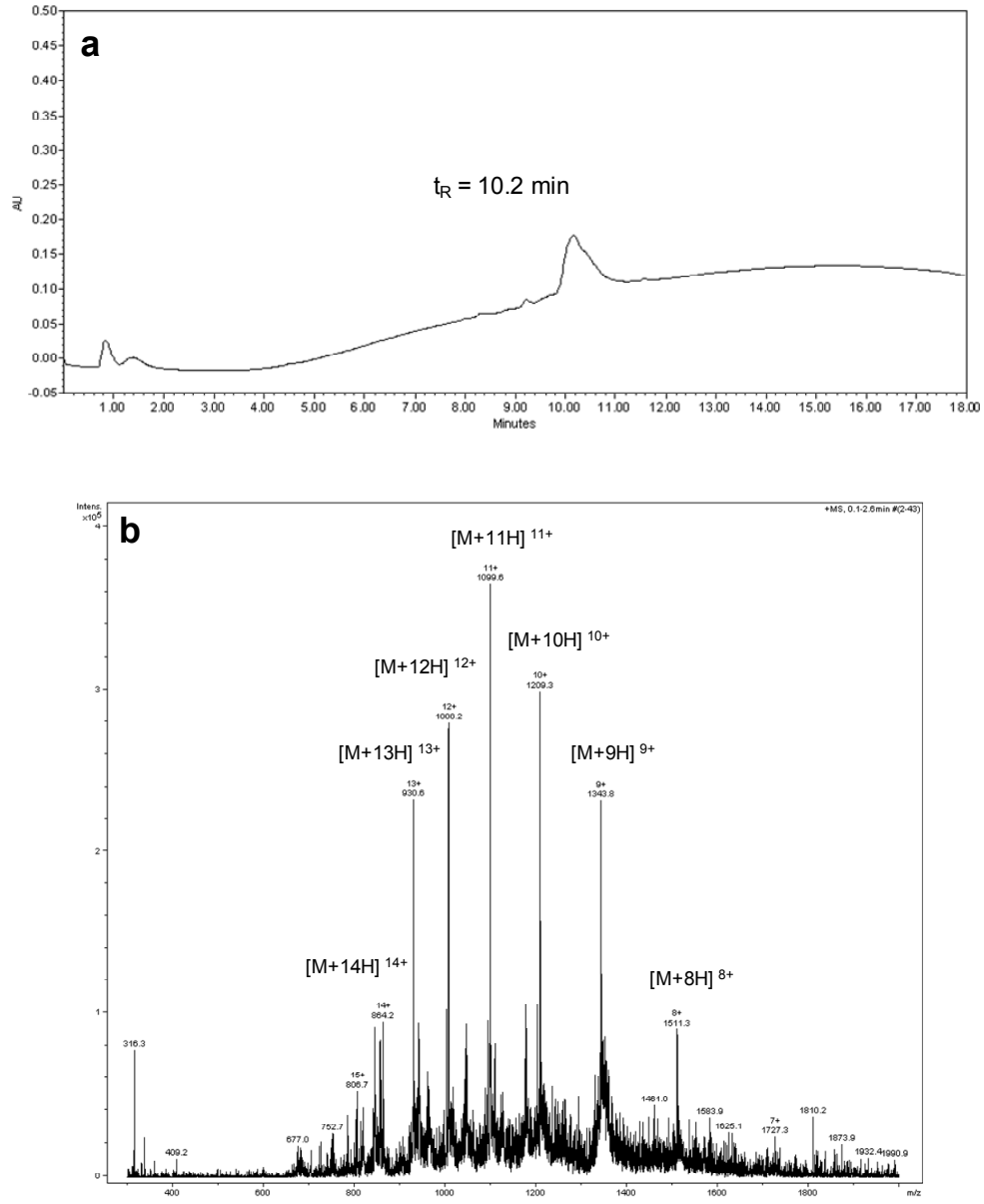


Figure S6. Characterization of compound **4Loop** by chromatography and mass spectrometry. RP-HPLC profile (a); ESI-MS analysis (b).

Kinetics studies. A concentration 25 µM of **4Loop**, **2Loop** and **2Lin** and 6 µM of **4Lin** were used for kinetic studies at 20°C. Fibril formation was monitored by the binding of Thioflavin T (10 µM), studying the fluorescence at 480 nm with excitation at 440 nm. The kinetic constants k_1 and k_2 were obtained using the Finke-Watzky (F-W) two-step mechanism of nucleation followed by autocatalytic surface growth.

$$A \xrightarrow{k_1} B$$

$$A + B \xrightarrow{k_2} 2B$$

Using this mechanism (where A is the initial monomer and B (catalytic) aggregated form of peptide edifices past the critical nucleus size) we can extract from experimental data, two constants, k_1, which represents the nucleation process and k_2, which represents the extension of the fibre (Table S1). This model can be mathematically translated by the following equations:

$$[A]_t = \frac{{k_1}/{k_2} + [A]_0}{1 + {k_1}/{k_2}.[A]_0 . \exp(k_1 + k_2.[A]_0).t}$$

or

$$[B]_t = [A]_0 - \frac{{k_1}/{k_2} + [A]_0}{1 + {k_1}/{k_2}.[A]_0 . \exp(k_1 + k_2.[A]_0).t}$$

	4Lin	**4Loop**	**2Loop**	**2Lin**
k_1 (min^{-1})	$160\times10^{-3} \pm 2\times10^{-2}$	$28\times10^{-3} \pm 4\times10^{-3}$	$28\times10^{-3} \pm 4\times10^{-3}$	$2.3\times10^{-3} \pm 4\times10^{-4}$
k_2 ($\mu M^{-1}.min^{-1}$)	$46\times10^{-3} \pm 5\times10^{-3}$	$6.1\times10^{-3} \pm 7\times10^{-4}$	$3.2\times10^{-3} \pm 4\times10^{-4}$	$3.6\times10^{-3} \pm 4\times10^{-4}$
$t_{1/2}$ (min)	2.5	8.5	12.6	270.2

Table S1. Rate constants and half-life time values from fitting kinetic data with the F-W two-step mechanism.

1. G. T. Dolphin, P. Dumy and J. Garcia, *Angew. Chem.-Int. Edit.*, 2006, **45**, 2699-2702.
2. G. T. Dolphin, M. Ouberai, P. Dumy and J. Garcia, *ChemMedChem*, 2007, **2**, 1613-1623.