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Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Journal Name

ARTICLE

Metal driven assembly of peptidic foldamers: formation of molecular tapes

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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In this article we report the use of a peptidic foldamer in the synthesis of supramolecular metal-organic structures. A peptidic ligand was designed and synthesised to adopt a canonical 3_{10} helix with pyridil ligands at each end, as confirmed by X-ray diffraction analysis. The combination of the synthesised peptide with silver salts in the adequate reaction conditions results in the production of coordination polymers. The materials thus produced have been analysed by means of SEM-EDS confirming the formation of long, regular, tape-shaped fibres that contain both the organic moiety and the metal atom. ATR FT-IR sprectroscopy suggests that the helical structure of the peptide was preserved within the fibre.

Introduction

In the last years there has been a rising interest in supramolecular processes to generate novel materials with interesting properties.[1] In this context, coordination chemistry offers a dynamic methodology that provides bond energies between the strong covalent bonds and the weak non-covalent interactions. Accordingly, coordination metal organic polymers and gels,[2] metal-organic frameworks (MOFs)[3] and supramolecular cages[4] have been described and they cover a broad range of applications, from molecular recognition[5] to supramolecular catalysis[6] and sensing.[7] To build such systems rigid scaffolds are needed and therefore usually aromatic, symmetrical abiotic ligands are employed. Whereas constraining the conformational space, together with a cautious control of coordination geometries, results in the synthesis of discrete structures,[8] the most common abiotic ligands have some drawbacks for their use in biological applications. Amino acids and other biologically relevant molecules have also been reported for the construction of supramolecular, metal-organic structures.[9] In particular, peptides can offer a suitable alternative for the construction of supramolecular assemblies[10] and can provide a huge variety in terms of functional groups, polarity, lipophilicity or charge but they also present serious disadvantages towards the constructions of supramolecular structures. Significantly, oligopeptides are conformationally flexible and this issue prevents their use to build scaffolds with big cavities as such systems would collapse. Nevertheless, some examples of crystalline metallopeptides can be found in the literature.[11] In addition, Fujita showed that the use of rigid, folded, helical, peptides can generate macrostructures by metal coordination giving rise to nanometre-sized channels[12] and impressive complex catenane structures.[13]

We hypothesized that the use of constrained foldameric structures could provide a new tool to construct novel metal-organic compounds with a peptidic backbone. With a more rigid core, the formation of supramolecular architectures should be possible.[14] We turned our attention to the oligomers of aminoisobutyric acid (Aib).[15] These compounds are known to adopt 3_{10} helical configurations stabilized by intramolecular CO···H-N hydrogen bonding (β-bend). The rigidity of the structure and regularity of the helical pitch provides well-defined distances and directions of suitable functionalities, providing a tool for the construction of supramolecular structures. This methodology found some applications in recognition and catalysis but to the best of our knowledge their use as molecular tools is still scarce.[16] Based on the literature reports and our own experience, we decided to investigate the synthesis of supramolecular structures built from the metal coordination of oligomers of Aib. To this aim we decided to introduce two pyridine ligands in the structure of the peptide, separated by two helix turns which would place the functional groups in an approximate average distance of 11.5 Å.[17] In order to favour helicity in the *C*-terminus, the peptide was capped with a methylamide while the *N*-terminus was kept as *tert*-butyl carbamate. Pyridyl ligand moieties were easily introduced by using the commercially available 4-pyridyl alanine to a central core of Aib_5. Herein we show the preparation and the X-ray crystal structure of peptide **4** in two different solvent systems and its coordination behaviour with silver salts to generate regular, self-assembled, molecular tape-shaped metal-organic fibres as evidenced by SEM-EDS analysis.

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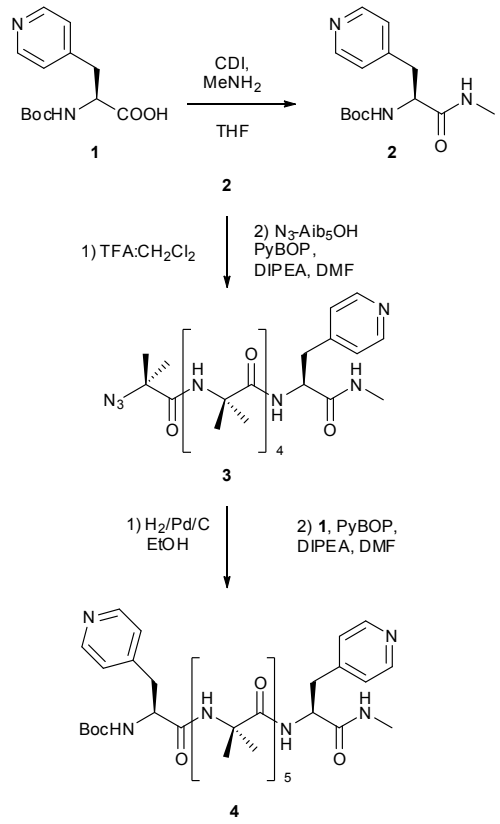
† Electronic Supplementary Information (ESI) available: Synthetic details for the formation of peptide **4**, NMR spectra of new compounds and SEM images of **4** with different silver salts and solvent mixtures are available. X-ray data for **4** (CIF) [CCDC numbers for the crystal structures: 1443789 and 1446622]
DOI: 10.1039/x0xx00000x



Scheme 1 Synthesis of peptide ligand **4**

Results and discussion

Peptide 4 was easily obtained following the synthesis described in Scheme 1. Crystals of **4** suitable for X-ray analysis were grown by slow evaporation of a concentrated solution in acetonitrile. Their X-ray diffraction analysis showed the expected formation of a canonical right-handed 3_{10}-helical structure (Fig. 1A, 1B); with 6 intramolecular N-H···O=C hydrogen bonds. As anticipated, the pyridyl residues point to the same direction in space (Fig. 1B), separated by two full helical turns. Thus, the nitrogen atoms in the pyridines are separated (in the conformation present in the crystallographic cell) by 14.4 Å. The molecules pack head-to tail in the helix direction through intermolecular hydrogen bonds and, interestingly, in the perpendicular axis through a water molecule bridge (Fig. 1C). With the peptide **4** in hand, we proceeded to investigate its behaviour in the presence of a metal atom. We chose Ag^+ as it has flexible coordination geometries and can form linear complexes that could lead to the formation of supramolecular cages or metal based polymers.[18] Importantly, silver complexes have shown interesting biological properties as antibacterial and anticancer agents.[19] We expected that by carefully controlling the stoichiometry, a limited amount of complexes would be formed.

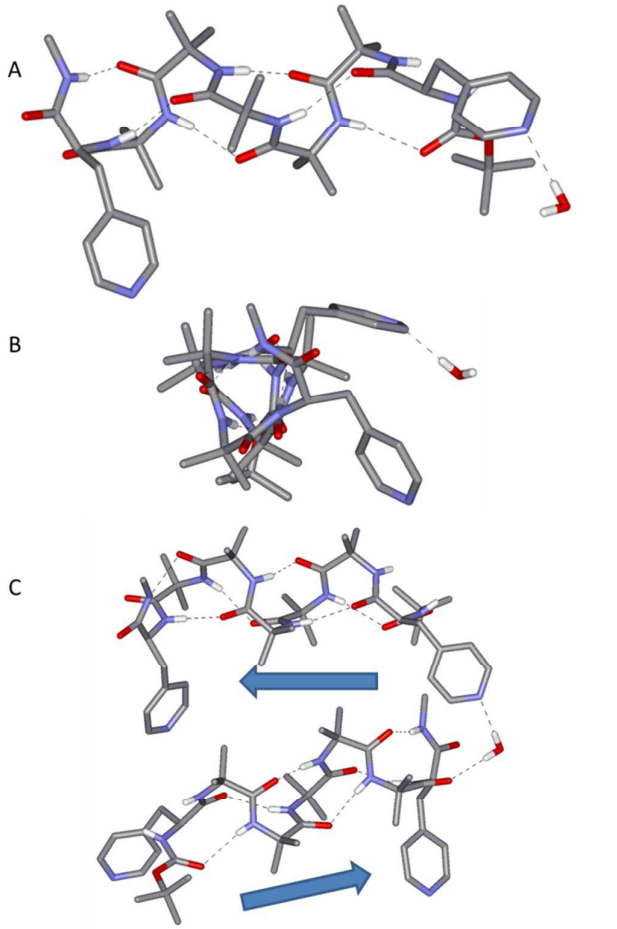


Fig. 1 Different views of the X-ray crystal structure obtained for peptide **4** highlighting the observed interactions. A) Helix side view. B) Helix top view showing the pyridine disposition. C) View along the crystallographic c axis showing the packing of antiparallel molecules through a water molecule. Non-polar H atoms have been omitted for clarity.

However, we were aware that even if 2:2 metal:ligand complexes were formed, two possible products could be formed depending on the relative orientation of the peptide moieties. Direct mixing of ligand **4** with silver salts in equimolar proportions in water:ethanol or water:THF mixtures resulted in the formation of amorphous precipitates. These precipitates remained insoluble upon heating as a sign of the formation metal-organic oligomers or polymers. Nevertheless, we were able to obtain microcrystalline fibres by slow diffusion of a sample of peptide **4** (30 mM) in ethanol into an aqueous solution containing the same concentration of a silver salt (Fig. 2a). Thus, the formation of a gel, fibrous material could be observed by naked eye after 3-4 days. We analysed this material by Scanning Electron Microscopy (SEM) which revealed the formation of moderately regular long, tape-shaped fibres. The fibre formation occurred independently of the counteranion employed (NO_3^-, BF_4^-, TfO^-, ClO_4^- were examined, Fig. 2).

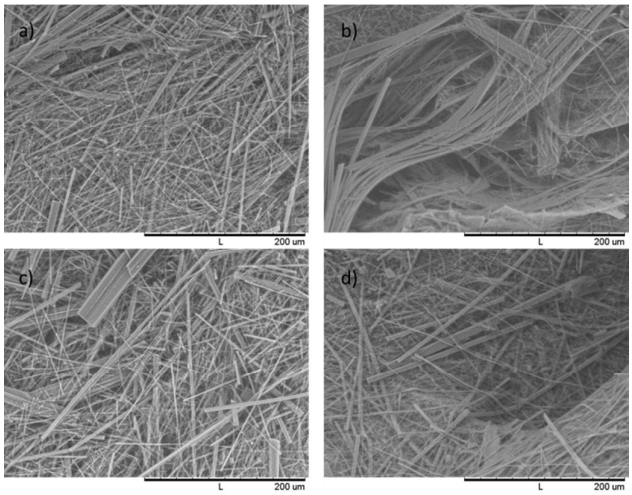


Fig. 2 SEM image of fibres formed by peptide **4** and a) $AgNO_3$; b) $AgBF_4$; c) AgOTf; d) $AgClO_4$.

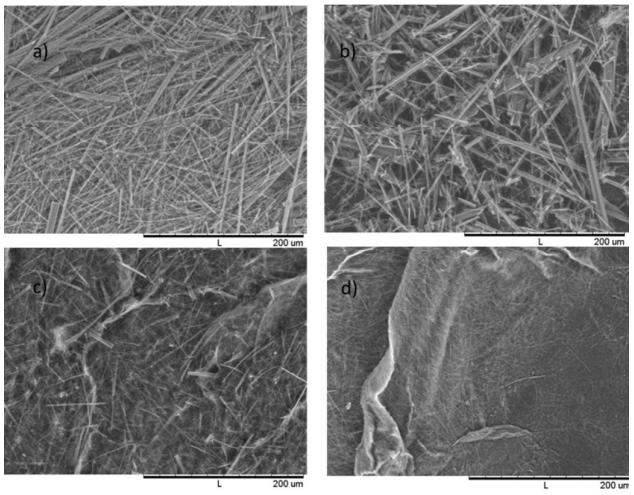


Fig. 3 SEM images of fibres formed by peptide **4** and $AgNO_3$ in different co-solvents: a) ethanol, b) THF, c) MeOH and d) DMF

The length of the supramolecular fibres varies from approximately 200 µm to 1 mm (Fig. ESI2-ESI5). Considering the structure obtained by ligand **4** alone, which shows stacks of peptides bonded by water molecules, and the SEM images obtained in the presence of Ag^+, we assumed that supramolecular metal-organic polymers were obtained. Thus, peptide residues would be linked by metal centres forming a network. Supramolecular metal-organic polymers have been widely studied[20] and, interestingly, silver-based polymers have found applications for example as antibacterial agents.[21] Replacing ethanol by other organic solvents generated fibrous material but with some differences depending on the solvent used (Fig. 2). THF resulted in more size dispersity with a larger amount of shorter tapes. Methanol produced a gel-like material with thinner and less defined fibres and a similar result was observed in DMF. Finally, the use of MeCN resulted in no fibre formation, most likely because acetonitrile is a strongly coordinating solvent which can compete with pyridine for the metal atoms (Fig. 4). This result suggested that the coordination of the pyridine nitrogen to the silver metal centre was needed for fibre formation.

The fibres were formed in a narrow concentration range, with no appreciable formation after 2 weeks for 15 mM samples (or below) and the formation of a precipitate over 60 mM. The fibres remained stable for a period of 1 month in the dark and showed no visible signs (SEM analysis) of degradation when exposed to light for a week (in ethanol:water mixture). To get a better insight in the fibre composition we performed a high-resolution SEM-EDS analysis of the fibrous material obtained using silver nitrate as metal source and peptide **4** (Fig. 4). In agreement with the previous analysis, tape-like fibres were observed. Thus, quite regular structures are formed with lengths comprised between hundreds of micrometres and one millimetre, with width between one and ten micrometres. Fibres are stacked in nearly parallel orientation forming bundles (Fig. 4d), probably caused by the direction of growth promoted by slow diffusion.

We also performed an EDS analysis which confirmed the presence of silver, together with C, N and O elements (Fig. 4e). Therefore, this analysis unambiguously showed that the fibres are formed by both the peptide ligand and the metal cation.

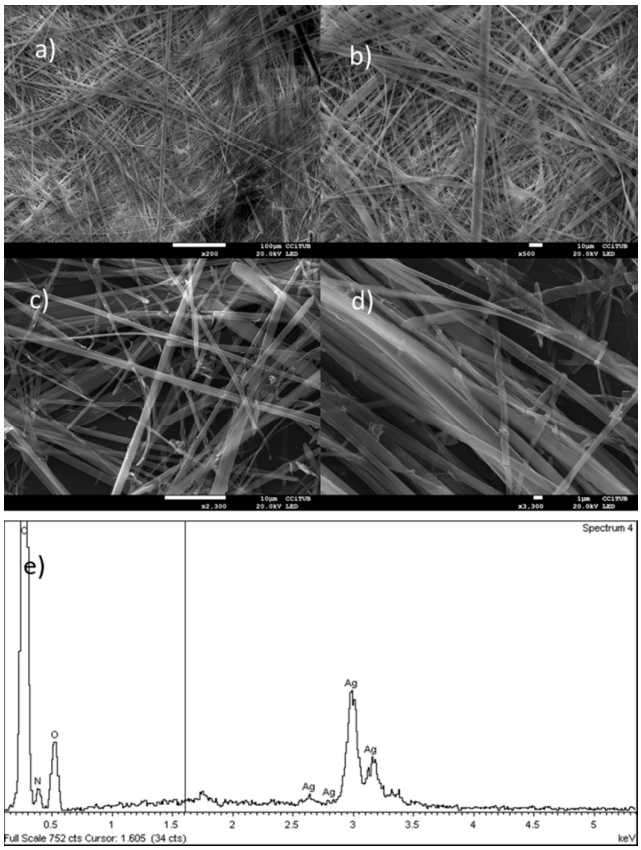


Fig. 4 a-d) HR-SEM images of the fibrous material obtained by slow diffusion of peptide **4** into a silver nitrate solution (30 mM each) after 4 days. a) Scale shows 100 µm, b), c) scale shows 10 µm, d) scale shows 1 µm. e) EDS analysis of the fibrous material confirming the presence of silver within the fibres.

We were intrigued to know the structure that peptide **4** would adopt in these fibres. As X-ray diffraction studies were not possible due to the lack of suitable crystal material we turned our attention to IR spectroscopy. Solid-state ATR-FT-IR spectrum for peptide **4** showed intense bands at 3287, 1650 cm^{-1} which show H-bonded NH-groups, strongly bonded C=O groups.[22] In addition a sharp band at 1530 cm^{-1} can be assigned to the Amide II band (Fig. 5a). In ethanol solution, the NH stretching band disappears and the position of the carbonyl band slightly moves to 1658 cm^{-1}, both phenomena in agreement with solvent competing with intramolecular H-bonds (see ESI). More interestingly, when the fibres were analysed by ATR-FT-IR spectroscopy, the position of the NH and CO stretching bands were unaltered with respect of those obtained from the solid sample of pure **4**, which indicates the preservation of the 3_{10} helical pattern in the metal-organic structure (Fig. 5b). This analysis confirmed therefore that the fibre is formed by both the peptide ligand and the metal cation with the peptide ligand maintaining its 3_{10} helical structure. Moreover, qualitative NMR experiments allowed us to conclude that metal coordination does not alter the 3_{10} helical structure. In a sample containing the peptide (10 mM) d_3-acetonitrile addition of $AgBF_4$ (10 mM) as silver source mainly results in the perturbation of the chemical shift of the pyridine protons (and to a lesser extend of some of benzylic protons), consistent with formation of a metal complex (see ESI). Although these conditions do not result in the formation of fibres they allowed us to study by solution NMR the effect of the metal complex formation in the peptide structure. Thus, importantly, titration experiments of this complex with d_6-DMSO showed that only two NH-protons move downfield with the addition increasing quantities of d_6-DMSO. This is consistent with the formation a 3_{10} helical structure as only two protons (the NH-Boc and the first NH-amide) are not forming part of intramolecular hydrogen bonds and, therefore, are exposed to the effect of adding DMSO. Overall, these NMR experiments further confirmed that the metal coordination does not alter the helical structure of the peptide ligand.

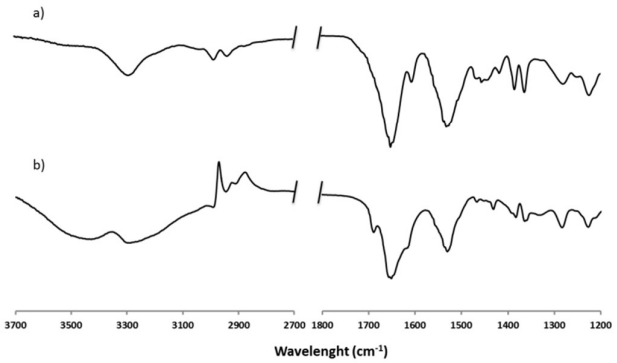


Fig. 5 Partial ATR-FT-IR spectra showing the most characteristic bands of: (a) compound **4**; (b) fibres grown in EtOH:water as described in the experimental section using $AgNO_3$ as silver source. The position of the amide bands remains unchanged in the fibre suggesting that the helical structure is preserved within the fibre.

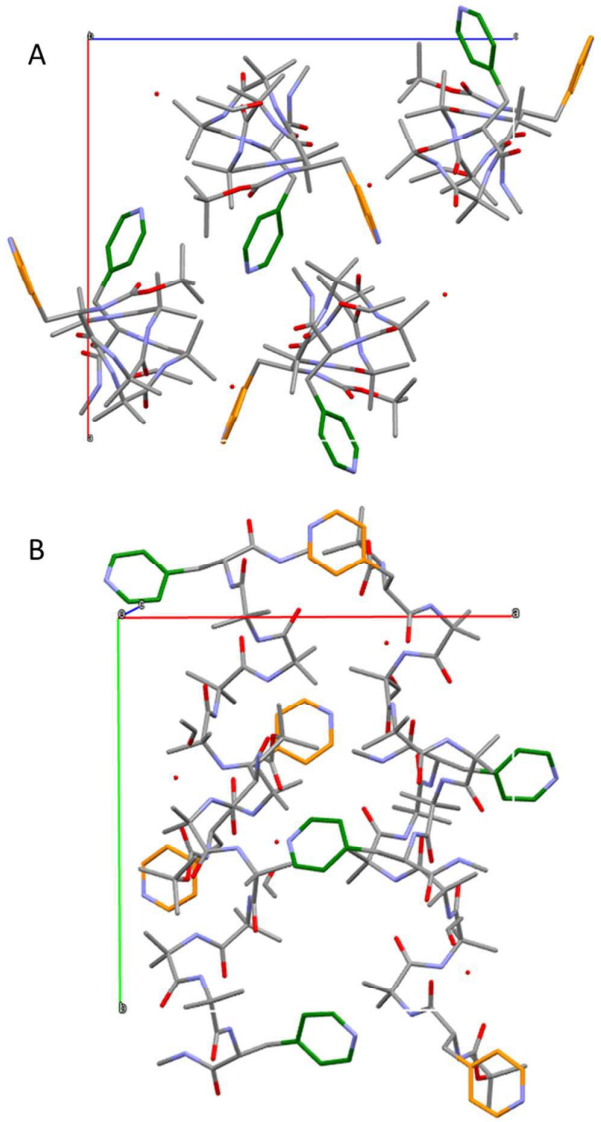


Fig. 6 X-Ray structure obtained by degradation of fibres formed by peptide **4** and silver nitrate. A) View along the b axis showing antiparallel peptides in the c axis. B) View from the c axis, antiparallel peptides are arranged in the a axis. For clarity, pyridine residues are displayed in different colours: orange in the *N*-terminus and green in the *C*-terminus, hydrogen atoms have been omitted.

Although the fibres remained stable for days, serendipitous degradation occurred when a loose capped vial allowed the evaporation of the solvent in the presence of oxygen and light.[23] Thus, hexagonal prism crystals were formed with the appearance of dark-brown metal depositions, probably due to the formation of oxidized silver species. X-ray diffraction analysis of the crystals confirmed the demetalation process as only ligand and solvent molecules (ethanol and water) were observed in the crystal. Interestingly, although the 3_{10} helical structure was conserved, a slightly different conformation (for the pyridine residues) and packing were obtained (Fig. 6). In the new crystal, the peptides are aligned head to tail (connected by an ethanol molecule via N-H…O and O-H…O

hydrogen bonds) in chains running along the b axis (See Fig. ESI9), and in an orthorhombic cell. Looking along the a-axis and the c-axis, the molecules are arranged in an anti-parallel way, a disposition that should be favoured by the peptide dipole-dipole interactions (Fig. 6).

To clarify whether the new crystal disposition was an effect of the solvent system or a consequence of the metal promoted self-assembly in the fibre, we crystallised the pure peptide **4** in wet ethanol. The crystals thus obtained were analysed by X-ray diffraction and the obtained crystal cell was the same as for the demetallated material, concluding that the conformation must be a consequence of the solvent and not of the assembly. However, this new disposition of the ligands allowed us to propose a tentative mechanism for fibre growth. Discrete peptide-silver complexes could be formed in different orientations in a dynamic equilibrium. Formation of offset complexes between adjacent layers would trigger fibre initiation in a disposition similar to that displayed in Fig. 5, where the metal ions could take the position of the coordinating solvent. Although this suggestion is somehow speculative it would provide a nucleation point from which the fibre could grow, probably in the diffusion direction. As a result, this metal-directed self-assembly process between helices would produce polymeric material that ultimately forms the observed fibres.

Conclusions

We have synthesised a helical peptidic ligand containing an oligomeric Aib_5 core and pyridyl residues that folds into a 3_{10} structure as evidenced by X-ray diffraction analysis of sigle crystals. The folded peptide is able to create supramolecular metal-organic structures by metal promoted self-assembly. The formed structures form polymeric fibres reasonably regular in size with lengths comprised from hundreds of micrometres to 1 mm in the most favourable solvent system. The counteranion employed seemed to have no effect in the fibre formation but the election of the solvent system was critical in order to have regular structures. The IR data collected suggest that the helical structure is conserved within the fibre while SEM-EDS analysis confirmed the presence of both the peptide and the metal in the fibre structure. Metal-organic polymers and metallogels[24] are becoming increasingly important thanks to their potential applications in a range of fields as diverse as catalysis or tissue engineering. Here we show how rigid peptides helices and metal coordination can be combined as design vectors to construct new supramolecular polymers. Applications of these and structurally related materials are under evaluation in our lab.

Experimental section

Materials and methods.

ATR FT-IR spectra were recorded in a Nicolet FTIR Avatar 360.

Low resolution SEM images were obtained in a Hitachi tabletop microscope TM-1000. Accelerating voltage 15 kV
High resolution SEM-EDS analyses were performed in a Jeol SEM J-7100F with an Inca 250 series EDS detector from Oxford Instruments.
X-ray crystallographic analysis Crystal Structure Analyses: Data were collected on a STOE IPDS II two-circle diffractometer with a Genix Microfocus tube with mirror optics using MoKα radiation (λ = 0.71073 Å). The data were scaled using the frame scaling procedure in the X-AREA program system.[25] The structure was solved by direct methods using the program SHELXL[26] and refined by full-matrix least-squares techniques using SHELXL. The H atoms bonded to N in compound 4 (grown from demetallation) were refined using a N-H restraint of 0.88(1). The distance O1E-C1E was restrained to 1.44 Å. The H atoms bonded to N in peptide **4** (grown in MeCN) were freely refined. All other H atoms were refined using a riding model.

Experimental details crystal formation and crystallographic information.

Suitable crystals for X-ray analysis of **4** were obtained by slow evaporation of a concentrated sample in MeCN. Crystals of 4 (grown from demetallation) in EtOH:Water mixture were obtained serendipitously after slow evaporation of a 0.5 cm-diameter tube containing fibres formed by the peptide 4 and $AgNO_3$ according to the procedure described bellow. The main crystallographic parameters are summarised in Table 1.

Table 1. Crystallographic information for the X-ray structures obtained for compound **4**.

	Compound **4** (**grown in MeCN**)	Peptide **4** (**grown from demetallation**)
Empirical formula	$C_{42}H_{64}N_{10}O_9$, H_2O	$C_{42}H_{64}N_{10}O_9$, C_2H_6O, H_2O
Formula weight	871.05	917.11
Crystal system	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	16.4496(6)	16.7243(10)
b (Å)	16.5567(7)	16.7433(9)
c (Å)	17.7879(7)	17.6543(10)
V (Å^3)	4844.6(3)	4943.6(5)
Z	4	4
D_{calcd} (Mg m-^{3})	1.194	1.232
μ (mm^{-1})	0.086	0.089
Reflections collected	62992	47952
Data/parameters/restraints	9108/592/0	9359/613/9
F(000)	1872	1976
R_{int}	0.0616	0.0732
R1, wR2 [I>2σ(I)]	0.0469, 0.1045	0.0841, 0.2063
R1, wR2 (all data)	0.0562, 0.1085	0.1140, 0.2243
Gof	1.052	1.095

Experimental procedure for fibre formation

In a typical experiment a solution of silver salt in water (200 µL, 30 mM) was placed in a 0.5 cm diameter tube. A buffer solution 1:1 of water and the solvent employed (150 µL) was added carefully without mixing the phases. Finally a 30 mM solution of peptide 4 in the solvent of choice was added (200 µL). The mixture was allowed to stand in the dark for 4 days; after this period the formation of a gel can be observed and the fibres were analysed by electron microscopy.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO, CTQ2012-38543-C03-03 and CTQ2015-70117-R) and Generalitat de Catalunya (2014 SGR 231). J.S. acknowledges MINECO (Ramon y Cajal contract) and EU (FP7-PEOPLE-2012-CIG-321659) for funding fellowship.

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