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New cylindrical peptide assemblies defined by extended parallel β-sheets†

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A new approach to non-covalent peptide-based nanotubular or rod-like structures is presented, whereby the monomeric units are preorganised into a β-strand geometry that templates the formation of an extended and unusual parallel β-sheet rod-like structure. The conformational constraint is introduced by Huisgen cycloaddition to give a triazole-based macrocycle, with the resulting self-assembled structures stabilized by a well-defined series of intermolecular hydrogen bonds.

Introduction

There is considerable interest in the design, synthesis, and exploitation of small peptide-like motifs that can self-assemble into higher levels of secondary structure, which mimic that found in peptides and proteins.[1–4] These extended structures and the associated processes leading to their formation, are central to understanding important diseases such as Alzheimer's[5–7] and also as a basis of biological probes to study protein structure and function. Peptide nanotubes and rods, resulting from the self-assembly of such motifs into extended β-sheets, are of particular fundamental interest in this context as unique molecular architectures and more practically as antimicrobial and chemotherapeutic agents.[8,9] Tubular structures are also found in Nature, *e.g.* the transmembrane channel proteins.[10,11]

Synthetic peptide nanotubes are typically constructed of cyclic peptides that stack by formation of a network of hydrogen bonds. The component cyclic scaffolds invariably consist of alternating sequences of an even number of D- and L-α-amino acids,[9] β-amino acids,[12,13] alternating α,γ-amino acids,[14,15] ∂-amino acids,[16] N-methylated amino acids,[17] non-peptide scaffolds[18–20] other such systems.[21,22] With a few exceptions,[12,23] these structures associate in an antiparallel fashion to give an extended cylindrical β-sheet that is stabilized by hydrogen bonding between backbone N–H and C=O groups that alternate up and down relative to the plane of the ring.

Here we present a new template-based approach to peptide-based nanotubes using a 'smart' scaffold designed to be constrained into a β-strand geometry, see Fig. 1. This species then nucleates the formation of an extended and unusual parallel β-sheet, with the component constraint directly involved in the self assembly. There is a strong thermodynamic preference for formation of an antiparallel β-sheet, over that of the analogous parallel geometry, in peptide-based nanotubes and also more generally in peptides and proteins.[24–29] Parallel β-sheets typically form from extended sequences that can be covalently linked by a tether to induce a turn and a suitable orientation to allow sheet formation.[26,30–35] By contrast, antiparallel β-sheets form with relatively short peptides of 12–25 residues.

The required β-strand conformational constraint was specifically introduced by linking the P1 and P3 side chains of a tripeptide with a 1,2,3-triazole-containing macrocycle such that the N- and C-termini are free for subsequent functionalisation. There are a few reports of cyclic peptides containing a

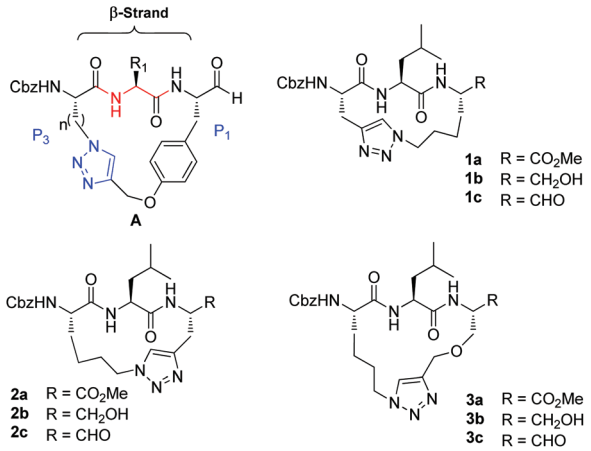

Fig. 1 Previously reported[37] macrocyclic peptidomimetic inhibitors (**A**) of calpain and new macrocycles (**1–3**), examples of which self-assemble into β-sheet stabilised nanotubular structures in solution and the solid-state.

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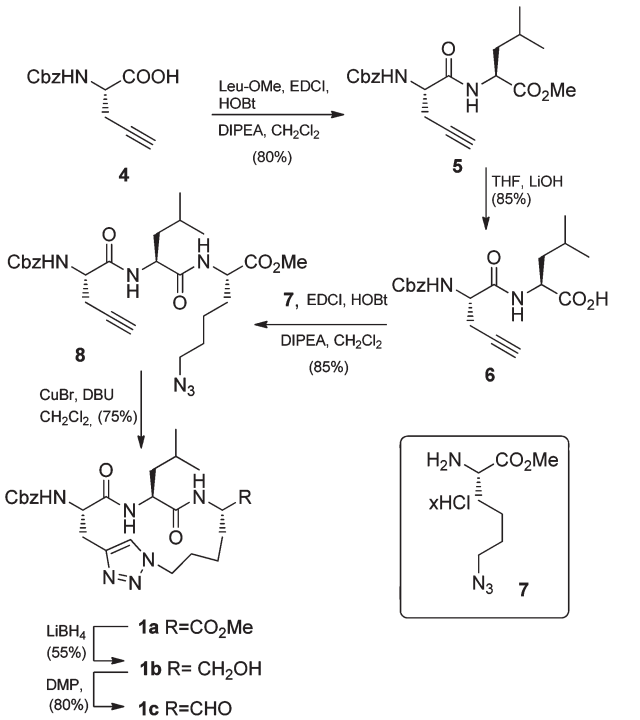
triazole that self-assemble, but in these the heterocycle does not participate in intermolecular interactions, the N- and C-termini are incorporated into the ring, and the cycle does not define a specific conformation.[36] Our new parallel β-sheet-based structure is stabilized by a well-defined series of hydrogen bonds involving the backbone amide and carbonyl groups and also the triazole ring within the macrocycle. We report the syntheses of these constrained β-strand macrocycles and also characterization of the resulting nanotubular structures derived from them by IR and NMR spectroscopy, X-ray crystallography, and ESI-MS. Derivatives **1a** and **2a** are also shown to form extended rod-like structures in the solid-state, consistent with bundles of nanotubes, when imaged by Scanning Electron Microscopy (SEM).

Results and discussion

We have previously reported triazole containing macrocycles as potent inhibitors of calpain, see **A** in Fig. 1.[37] While the component cycle constrains the peptide backbone into a β-strand conformation that is known to favour active site binding to the protease,[38,39] these structures do not appear to self assemble. A second and new series reported here lacks the aryl group in the macrocycle (see **1**, **2** and **3**) and is hence sterically less congested and also less hydrophobic. The new structures remain constrained into a β-strand geometry as revealed by IR, NMR and X-ray analysis, as discussed below. The critical observation is that these new macrocycles now self assemble into an extended and unusual parallel β-sheet based nanotube through a well defined network of hydrogen bonds.

A representative synthesis of the macrocycles (**1a**, **1b**, and **1c**) is shown in Scheme 1, with complete details on all compounds provided in ESI.†[37] In particular, the tripeptide **8** prepared from L-propargylglycine[40] by standard peptide coupling sequence as shown in Scheme 1, was cyclized in the presence of CuBr in CH_2Cl_2 to give the key macrocyclic ester **1a**. This was reduced with lithium borohydride to give the macrocyclic alcohol **1b**, which was purified by reverse phase HPLC. Oxidation of this alcohol, with Dess–Martin periodinane (DMP), then gave the macrocyclic aldehyde **1c** (Scheme 1).



Scheme 1 Synthesis of the β-strand template.

X-ray crystal structure analysis

The formation of a β-sheet derived nanotube was identified by X-ray crystallography. The 15-membered macrocycle **1a** crystallized from a chloroform–methanol solution in the orthorhombic space group $P2_12_12_1$ with four complete molecules in the unit cell.[41] The X-ray crystallographic structure of **1a** (see Fig. 2) revealed that the component macrocycle adopts a β-strand conformation (Φ = −125.2° and Ψ = 122.6°) in the solid-state, which remarkably stacks in a parallel fashion to form an extended nanotubular structure as shown in Fig. 2b–f. The tripeptide sequence in the macrocycle presents a planar β-strand backbone, with the carbonyl and NH groups alternating up and down to allow intermolecular hydrogen bonding, see Fig. 2a.

The component triazole-H hydrogen bonds to the N_2 and N_3 of an adjacent triazole ring to define the unusual parallel β-sheet. Fig. 2b–d depict the resulting open ended tubular structure that has an average diameter of ~6.0 Å (0.6 nm). The NH···O=C hydrogen bonds have bond lengths and angles that are typical for a self-assembled parallel β-sheet ($D_{NH\cdots O}$ = 2.01–2.11 Å; d_{NO} = 2.86–2.98 Å; $angle_{NO}$ = 163.1–171.1°), while the bifurcated hydrogen bonds of the triazole that support this nanotubular structure are slightly longer ($D_{CH\cdots N}$ = 2.57, 2.65 Å; d_{CN} = 3.21, 3.27 Å; $angle_{CN}$ = 125.3, 123.7°). The interior of the extended peptide nanotube is hydrophilic with the inclusion of the backbone amides and N_2–N_3 of the triazoles. By comparison, the exterior of the structure presents the exposed leucine side chains and the Cbz groups and is hence more hydrophobic in character. The hole through the nanotube is small, with an average internal diameter of ~3 Å (taking into account the van der Waals radii), and as such is free of solvent molecules.

The crystal structure also reveals that the individual peptide cylindrical columns are further packed into dimeric bundles defined by edge-to-face CH···π contacts involving the Cbz groups as shown in Fig. 2e and f.[42] The C–H···$\pi_{centroid}$ distance is 2.99 Å, while the closest C–H···C_{aryl} contact is 2.69 Å. The crystal packing is completed by association of the bundles into a herringbone-type arrangement in the *bc* plane.

NMR analysis

The ^{1}H NMR spectral data for **1a–c**, **2a–c** and **3a–c** in DMSO-d_6 are consistent with all these macrocycles adopting a β-strand conformation in solution. In particular, the $^3J_{NHC\alpha H}$ coupling

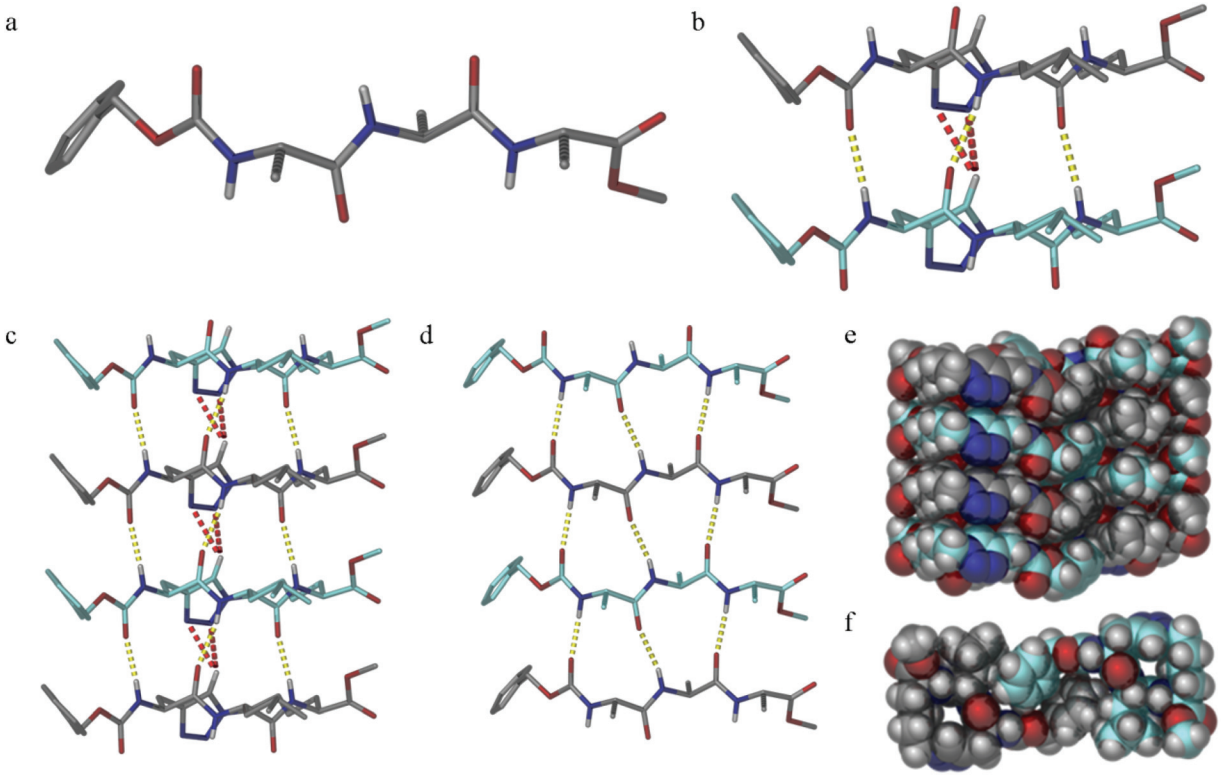


Fig. 2 (a) Depiction of the β-strand backbone of **1a** with the triazole containing macrocyclic linkage omitted for clarity. (b) A view of the intermolecular hydrogen-bonding observed between two macrocycles of **1a** with the hydrogen atoms of the side chains omitted for clarity. Peptide backbone hydrogen bonds are shown as yellow dashed bonds and the triazole hydrogen bonds as dashed red lines. (c) A representation of the structure of **1a** showing the nanotubular structure. (d) The structure of **1a** showing extended β-sheet conformation, with the triazole containing macrocyclic linkage omitted for clarity. (e) Side view of the dimerisation of the nanotubular structures in the crystal. (f) Top view of the dimerised nanotubes in a space-filling representation.

constants were observed to be in the range 8.4 to 9.3 Hz. This corresponds to literature values of 8.0 to 10.0 Hz for an Φ torsion angle of ≈−120° and hence the all-trans backbone conformation of a β-strand geometry (see Table 1).[43,44] ROESY spectra of **1a**, **2a**, **3a** revealed characteristic cross peaks between C_αHi and (i + 1NH), βHi and (i + 1NH), NHi and (i + 1NH). This is also consistent with a flat β-strand conformation for the peptidic backbone that allows intermolecular hydrogen bonding between the two parallel aligned macrocycles (see ESI†).[45–47]

Further evidence for intermolecular hydrogen bonding[48,49] and hence β-sheet formation arises from the observation that the amide hydrogen atoms in the ^{1}H NMR spectrum of **1a** (in 20% DMSO/CD_3OD) did not exchange over one week (see ESI†).

Table 1 $^3J_{NHC\alpha H}$ coupling constants[a]

Compound	P1	P2
1a	9.1	8.4
1b	ND	8.4
1c	8.7	8.4
2a	9.0	8.9
2b	9.3	9.1
2c	8.7	8.9
3a	8.4	8.7
3b	ND	8.6
3c	8.4	8.8

[a] Coupling constants determined in DMSO-d_6. ND not determined because of overlapping resonances.

IR analysis

Solid-state FT-IR spectra of **1a–c**, **2a–b** and **3a–c** revealed bands at 1635–1641, 1532–1537, and 3276–3302 cm^{-1} that correspond to the amide I, amide II and amide A stretches (see Table 2). These signals are once again characteristic of a β-sheet geometry for the macrocyclic nanotubes.[50,51] These compare favourably with literature values for a β-sheet structure with frequencies in the range 1612–1640 cm^{-1} for the amide I stretch.[51] More importantly, higher frequency bands

Table 2 Characteristic solid-state FT-IR bands (cm^{-1})

Compound	Amide I	Amide II	Amide A
1a	1641	1532	3292
1b	1637	1535	3281
1c	1636	1535	3280
2a	1638	1536	3277
2b	1634	1536	3279
3a	1635	1534	3302
3b	1634	1534	3287
3c	1634	1529	3282

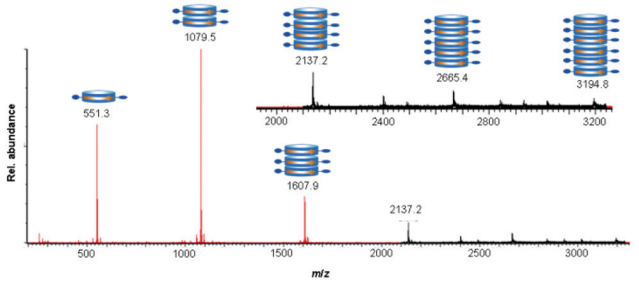


Fig. 3 ESI spectrum of compound **2a** showing the formation of oligomeric assemblies in the gas phase [M + Na].

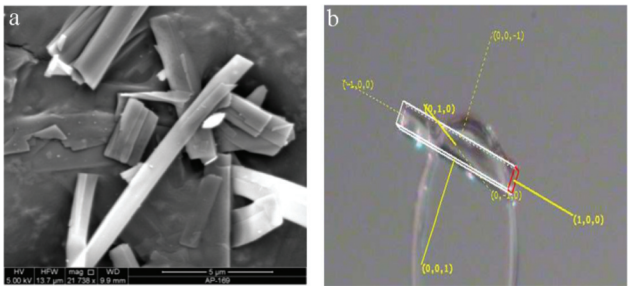


Fig. 4 (a) An SEM image of the rod-like assemblies of **1a**. (b) A view of the crystal of **1a** used for the structure determination and the Miller indices of the faces.

in the range 1530–1550 for the amide II stretch are consistent with a parallel β-sheet.[51] The NH stretching frequencies in the range 3281–3302 cm^{-1} are good evidence of the strongly hydrogen bonded and ordered nanotubular structure for all compounds.[50,51] As representative examples, the macrocycles **1a**, **2a** and **3a** gave the same characteristic IR bands at 3281, 1637–1638, 1539–1545 cm^{-1} in solution (see ESI†).

Remarkably, an ESI mass spectrum of **2a** (1 mM solution in 9 : 1 MeOH : DMSO) gave rise to characteristic peaks consistent with the monomeric, dimeric, trimeric, tetrameric, pentameric, and hexameric species, see Fig. 3. Compounds **1a** and **3a** also gave peaks for the corresponding oligomeric species under the same conditions (see ESI S24†). The observation of higher aggregates by mass spectrometry supports the formation of an extended nanotubular structure that is defined by the intermolecular hydrogen bonding of the β-sheet structure in solution and in the gas phase.

Scanning electron microscopy (SEM) images of samples of **1a**, revealed thin, well defined rod-like structures of 4–8 μm length and about 400–600 nm diameter as shown in Fig. 4 for **1a**. Face indexing of the crystal used for single crystal X-ray structure determination revealed that the long axis of the needle-shaped crystal coincides with the *a*-axis and the direction of growth of the nanotubular structure (see ESI†). Thus, the crystal form observed by SEM is consistent with extended bundling of the nanotubular structures.[52,53]

In conclusion, we have described the design, synthesis, and characterization of a new class of tri-peptide-based macrocycle constrained into a β-strand by component 1,2,3-triazole that links the P1 and P3 side chains of natural amino acids. NMR and FT-IR spectroscopy, mass spectrometry and X-ray diffraction data reveal that these macrocycles are intermolecularly hydrogen bonded to form parallel β-sheets and an extended nanotubular structure. The interior of these first-generation nanotubes is too small for host guest chemistry, although the synthetic approach and ideas employed provide scope for the formation of larger component macrocycles. Importantly, both termini of the peptidomimetic are exposed and available for further functionalisation, which is not the case for most of the existing peptide-based nanotubular structures. As a first example, we prepared the C-terminal aldehyde derivatives (**1c**, **2c** and **3c**) of the core template as shown in Scheme 1. These structures are potent inhibitors of calpain-II (IC_{50} = 355 nM, 582 nM, and 697 nM respectively), a result consistent with the macrocyclic constraint of these structures defining a β-strand geometry as is required for protease binding. Structures such as these may be of use in drug delivery and formulation applications. The work presented here defines a new approach to self assembled peptide-based nanotubular structures, whereby the monomeric units are themselves constrained into a β-strand geometry that templates the formation of an extended β-sheet geometry.

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