Large-diameter self-assembled dimers of α,γ-cyclic peptides, with the nanotubular solid-state structure of *cyclo*-[(L-Leu-D-^{Me}*N*-γ-Acp)$_4$-]·4CHCl$_2$COOH†

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Dimeric nanotube segments with pore diameters of up to 17 Å have been obtained by self-assembly from new α,γ-cyclic peptides.

Recent years have seen increasing interest in the use of porous materials in technological fields such as the storage of gases and liquids, energy conversion, and catalysis.[1] For these applications it is desirable to be able to tailor pore sizes and pore surfaces to each specific application. A class of porous material that in principle lends itself to easy control of pore size is a solid-state array of self-assembled peptide nanotubes (SPNs).[2] SPNs, which also have biomedical applications,[3] consist of self-assembling stacks of flat cyclic peptides (CPs), and their pore size depends exclusively on the size of these CPs.[2–4] However, the conformational flexibility of large CPs hampers their adoption of the flat conformation required for stacking, and has hitherto prevented the formation of SPNs using CPs with more than 36 backbone atoms and an internal diameter of 13 Å.[4]

With a view to favouring adoption of the required flat conformation, we have recently been working with CPs in which α-amino acids alternate with either (*cis*)-3-aminocyclohexanecarboxylic acid (γ-Ach) or (*cis*)-3-aminocyclopentanecarboxylic acid (γ-Acp) (Fig. 1, **1–3**).[5] The cycloalkane rings of these α,γ-CPs not only direct a hydrophobic, functionalizable methylene towards the interior of the CP ring (thus allowing manipulation of the behaviour of the cavity of α,γ-CP-based SPNs), but also ensure the flatness and rigidity of the cycloalkane segments of the CP backbone. The success of this strategy is attested to by the large association constants of some of the α,γ-CPs we have used (which for convenience have substituents that limit their stacking to the dimer stage).[6] Although we initially worked with γ-Ach-based α,γ-CPs, we began to use their γ-Acp analogues when we realized that γ-Acp is easily obtained from Vince's lactam[7,8] and, more importantly, that the angle defined in the plane of the CP ring by the C–N and C–C(O) bonds radiating from the cycloalkane ring is wider for γ-Acp than for γ-Ach, 140° as against 120° (Fig. 1). This makes γ-Acp more suitable for the construction of large α,γ-CPs.

In previous work[7] we confirmed that 6-residue γ-Acp-based α,γ-CPs, like their γ-Ach-based analogues, form stable dimers, and also that they can form even more stable heterodimers with γ-Ach-based α,γ-CPs. Here we describe the synthesis of 8-, 10- and 12-residue γ-Acp-based α,γ-CPs with backbones containing between 32 and 48 atoms, their high-affinity association in dimers, and the nanotubular array formed by at least one of them in the solid state.

Cyclic octapeptide **3b** was first prepared by solution-phase methods from the linear peptide Boc-[(L-Leu-D-^{Me}*N*-γ-Acp)$_4$-]-OFm, which was synthesized as described elsewhere.[6,7] TFA treatment of the linear peptide, followed by removal of the fluorenylmethyl group with piperidine and subsequent reaction with TBTU, gave the desired CP in 76% yield. Interestingly, a higher yield was obtained by condensation/cyclization of the linear tetrapeptide H-[(L-Leu-D-^{Me}*N*-γ-Acp)$_2$-]OH with TBTU and DIEA in DCM: although at peptide concentrations <0.2 mM the major product was tetrapeptide **1b**, at 5 mM formation of the 8-residue CP **3b** was almost quantitative.

In the ^{1}H NMR spectrum of **3b** in chloroform, the formation of dimer **D3b** is reflected by the downfield shift of the Leu NH signal. Furthermore, the fact that the location of this signal, 8.33 ppm, remains constant at concentrations down to 1×10^{-4} M, even in spite of addition of up to 50% of methanol to the solvent or heating to 323 K, shows that the association constant for dimer formation must be larger than 10^5 M^{-1}. The FTIR spectrum in chloroform shows the features that are characteristic of the β-sheet-like hydrogen bonding of α,γ-CP dimers,[5–7] having amide I, II$_{ii}$ and A bands at 1627, 1540 and 3309 cm^{-1}, respectively.

The CPs *cyclo*-[(D-Leu-L-^{Me}*N*-γ-Ach)$_5$-] (**4a**), *cyclo*-[(D-Leu-L-^{Me}*N*-γ-Acp)$_5$-] (**4b**) and *cyclo*-[(D-Phe-L-^{Me}*N*-γ-Acp)$_6$-] (**5**) were prepared by cyclization with TBTU from 1 mM solutions of the corresponding linear deca- or dodecapeptide in dichloromethane. The MS spectra of the γ-Acp-based CPs **4b** (yield 75%) and **5** (yield 85%) showed the expected peaks at *m*/*z* 1191 and 1634, respectively, and their NMR spectra reflect high symmetry in accordance with the desired flat conformations, each showing just one set of α-amino acid signals and one set of γ-Acp signals. The downfield shifts of their amide protons to 8.42 ppm (**4b**) or 8.61 ppm (**5**) suggest their involvement in dimerizing hydrogen bonds, and the formation of dimers **D4b** and **D5** was likewise supported by the amide I, II$_{ii}$ and A bands in their FTIR spectra at 1627, 1534 and 3313 cm^{-1} (**4b**) or 1623, 1525 and 3315 cm^{-1} (**5**). As in the case of **3b**, the association constant was shown to be at least 10^5 M^{-1} by the NMR signal of the amide proton remaining unaltered at concentrations down to 1×10^{-4} M even in spite of addition of up to 50% of methanol to the solvent or heating to 323 K.

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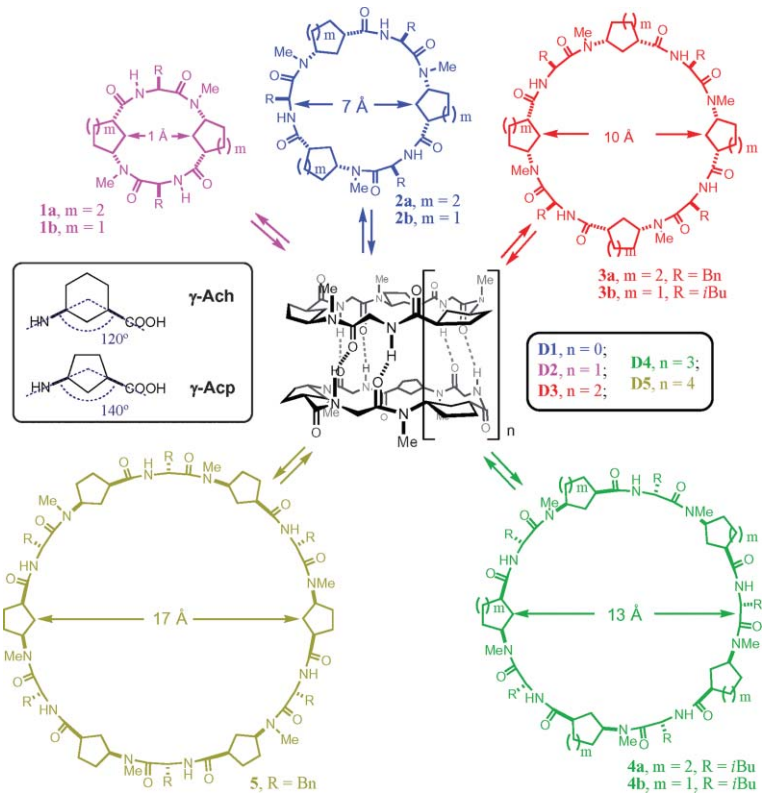

Fig. 1 Structures of α,γ-CPs rigidified by cycloalkane γ-amino acids (**1–5**), and the corresponding dimers (**D1–D5**). Inset left: CP backbone angles defined in the CP plane by cyclohexane and cyclopentane.

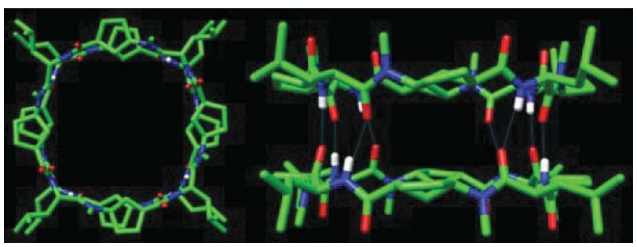

Fig. 2 Top (left) and side view (right) of crystal structure of **D3b** dimers obtained from the crystal structure.

Unlike those of **4b** and **5**, the NMR and FTIR spectra of the γ-Ach-based CP **4a** do not support the occurrence of dimer formation. Instead, the complexity of the ^{1}H NMR spectrum in $CDCl_3$ suggests the presence of a mixture of slowly interconverting conformers. This confirms that γ-Acp is more suitable than γ-Ach for the formation of CPs capable of forming SPNs with large pore diameters.

Conclusive proof of the dimerization of **3b** was provided by X-ray crystallography of the colourless prismatic crystals of **3b**·4$CHCl_2COOH$‡ that were obtained from a solution of this CP in tetrachloroethane (the dichloroacetic acid presumably[9] having been formed by light-induced oxidation of the solvent). The asymmetric unit of these crystals comprises four discrete moieties: two L-Leu-D-^{Me}*N*-γ-Acp units, and two $CHCl_2COOH$ molecules. Rotation around a fourfold symmetry axis generates another six $CHCl_2COOH$ molecules and two molecules of **3b** that together form a drum-shaped dimer (Fig. 2), each face of which is hydrogen-bonded to four of the dichloroacetic acid molecules. The component **3b** monomers are essentially flat and squarish, with C_α(Leu)···C_α(Leu) distances of approximately 13.4 and 13.6 Å and van der Waals diameters [$H_{\beta eq}$(Acp)···$H_{\beta eq}$(Acp)] of 6.1 and 6.2 Å, and in the dimers they are linked in antiparallel fashion by a β-sheet-like array of eight hydrogen bonds with N···O distances of 2.93 and 2.92 Å. In the crystal lattice the dimers are oriented with their 4-fold axes in the *c* direction, with the four dichloroacetic acid molecules on one face of one dimer interdigitating with the four on the adjacent face of the next (Fig. 3). This arrangement creates a nanotube formed by **D3b** units and dichloroacetic acid octets, which alternate along the *c* axis with a period of about 16.5 Å.

Solutions of CPs **4b** and **5** in tetrachloroethane afforded good-looking crystals. Unfortunately, we have not been able to resolve these structures, possibly because the large lumens of **D4b** and **D5** must allow considerable disorder among solvent molecules trapped inside them.

To sum up, we have prepared new, γ-Acp-based α,γ-CPs that in spite of their large diameters readily adopt flat conformations and form highly stable dimers through β-sheet-like series of hydrogen bonds. The ability of dimers of **3b** to form nanotubular solid-state arrays was confirmed crystallographically after a solution of **3b** in tetrachloroethane yielded crystals in which **D3b** co-assembled with rings of dichloroacetic acid molecules thought to have been formed by light-induced oxidation of the solvent. The nanotubes derived from this novel crystallization methodology may have applications in fields such as separation technology or gas storage.

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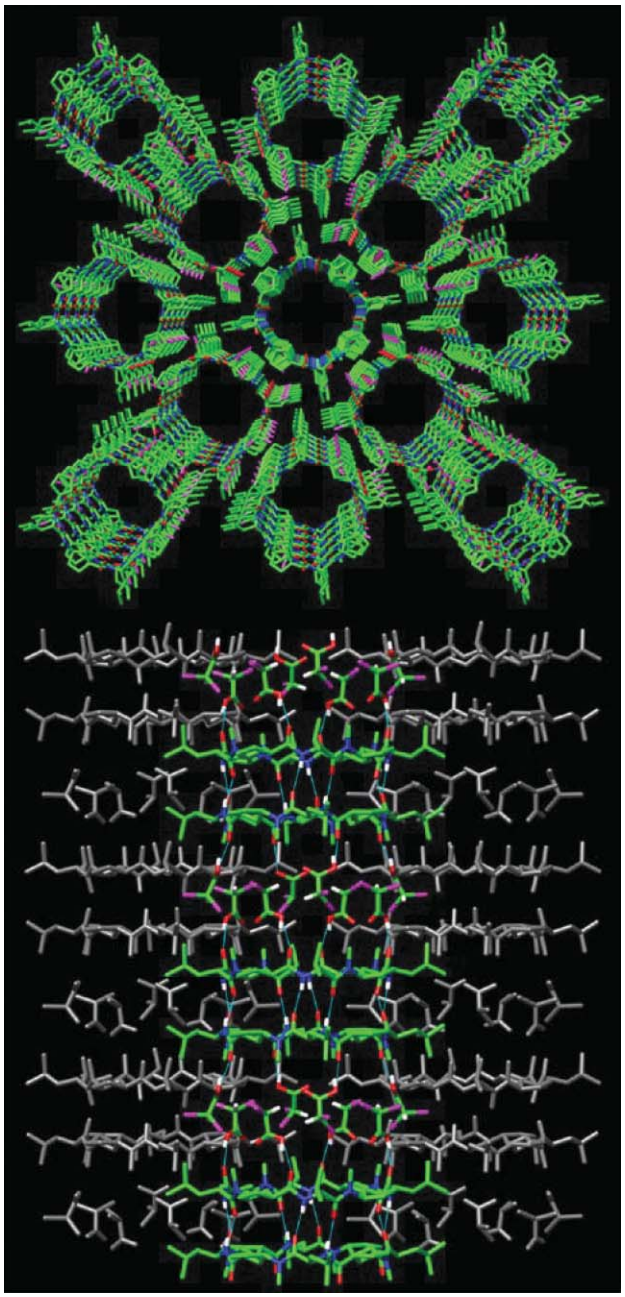

Fig. 3 Top and side view of nanotubes packing in the crystal structure.

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Notes and references

‡ Crystal structure data for **3b**: model formula $C_{52}H_{88}N_8O_8{\cdot}4C_2H_2Cl_2O_2$, M_r = 1469.05, tetragonal, $I\bar{4}$, a = 23.6648(9), b = 23.6648(9), c = 16.5051(13) Å, V = 9243.2(9) Å^3, D_c = 1.056 g cm^{-3}, Z = 4, T = 120.0(1), μ = 0.296 mm^{-1}, $F(000)$ = 3104, GOF = 1.07. Of 34689 total reflections collected, 7868 were unique (R_{int} = 0.0388). $R1$ ($wR2$) = 0.0736 (0.2042) for 424 parameters and 5748 reflections [$I > 2\sigma(I)$]. The intensity data were collected on a BRUKER Smart CCD diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). Absorption corrections were performed by using the SADABS program. The structure was solved by direct methods (SIR97) and refined by full-matrix least squares on F^2 using the SHELXTL-97 program package. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were included in the structure factor calculation at idealized positions by using a riding model. The void volume per unit cell, 2209.0 Å^3, is divided in two equivalent channels centered at (0, 0, 0) and (1/2, 1/2, 0). Attempts to model solvent molecules in these voids suggested the presence of 7 water and 1 methanol molecules per asymmetric unit, but the model refined poorly and these molecules were accordingly removed, their contribution to the scattering factors being taken into account with PLATON/SQUEEZE. A total of 710 e$^-$ were removed per unit cell (355 e$^-$ per cavity), corresponding to approximately 28 water and 4 methanol molecules per cavity (352 e$^-$). CCDC 635857. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b703659k

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