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The Smallest α,γ-Peptide Nanotubulet Segments: Cyclic α,γ-Tetrapeptide Dimers

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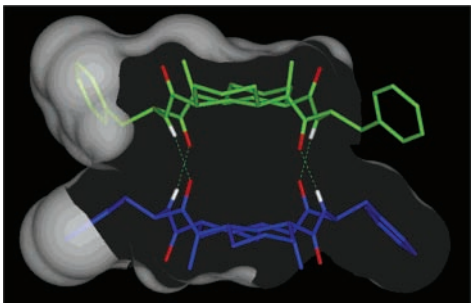
ABSTRACT



Cyclic tetrapeptides in which α-amino acids alternate with *cis*-3-aminocycloalkanecarboxylic acids dimerize by forming hydrogen bonds between their α-faces but not between their γ-faces, establishing the minimal structural requirements for the novel α,γ-peptide hybrids SPN.

The formation of nanostructures with technological or biomedical applications or promise often relies on their self-assembly through the spontaneous interactions of their component molecules.[1] Exploitable types of interactions that are found in nature include the hydrogen bonding responsible for the formation of β-sheets in proteins. The properties of this kind of hydrogen bonding have been studied relatively little (in comparison with those of other common protein secondary structures) because of the poor solubility of peptide models,[2] but β-sheetlike hydrogen bonding is nevertheless being used as the constructional mechanism for preparation of self-assembling peptide nanotubes (SPNs), a growing class of nanostructures in which flat cyclic peptides are stacked at a peptide-to-peptide distance of about 4.73 Å. Because of their ready functionalization, these nanotubular structures hold out enormous promise for transport of molecules or ions and as catalysts or molecule containers.[3] In particular, SPNs formed of α,γ-CPs (cyclic peptides in which a D- or L-α-amino acid alternates with, respectively, an L- or D-*cis*-3-aminocycloalkanecarboxylic acid γ-Aca)[4] can, in principle, be easily functionalized at their inner faces by substitution on C2 of the cycloalkane ring (Figure 1, top right).

The cyclic peptides constituting SPNs are flat because of the chiralities of their amino acids, and they stack together because these chiralities also orient their C═O and N−H

(1) For recent reviews of supramolecular chemistry and self-assembly, see: *Science* **2002**, *295*, 2395−2421 and *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4762−5188 (special issues).

(2) (a) Sneer, R.; Weygang, M. J.; Kjaer, K.; Tirrell, D. A.; Rapaport, H. *ChemPhysChem.* **2004**, *5*, 747−750. (b) Nesloney, C. L.; Kelly, J. *Bioorg. Med. Chem.* **1996**, *4*, 739. For a recent review on the design and synthesis of β-sheet models, see: (c) Longhlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, *104*, 6085−6118.

(3) (a) Brea, R. J.; Granja, J. R. Self-Assembly of Cyclic Peptides in Hydrogen-Bonded Nanotubes. In *Dekker Encyclopedia of Nanoscience and Nanotechnology*; Schwarz, J. A., Contescu, C. I., Putyera, K., Eds.; Marcel Dekker: New York, 2004, pp 3439−3457. (b) Patzke, G. R.; Krumeich, F.; Nesper, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 2446−2461. (c) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988−1011. (d) Ajayan, P. M.; Zhou, O. *Z. Top. Appl. Phys.* **2001**, *80*, 391−425.

(4) For six amino acid α,γ-CPs, see: (a) Amorín, M.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 2844−2845. (b) Amorín, M.; Villaverde, V.; Castedo, L.; Granja, J. R. *J. Drug Delivery Sci. Technol.* **2005**, *15*, 87−92. For eight amino acid α,γ-CPs, see: (c) Amorín, M.; Castedo, L.; Granja, J. R. *Chem.—Eur. J.* **2005**, *11*, 6539−6547. For α,γ-CP heterodimers formation, see: (d) Brea, R. J.; Amorín, M.; Castedo, L.; Granja, J. R. *Angew. Chem., Int. Ed.* **2005**, *44*, 5710−5713.

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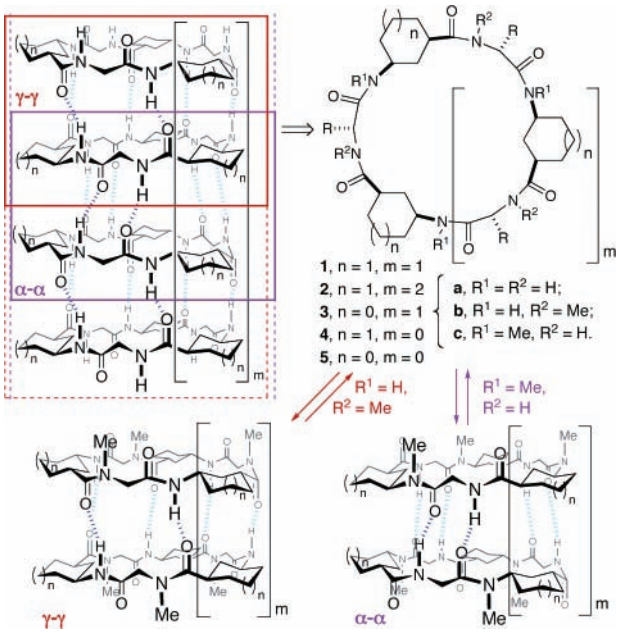


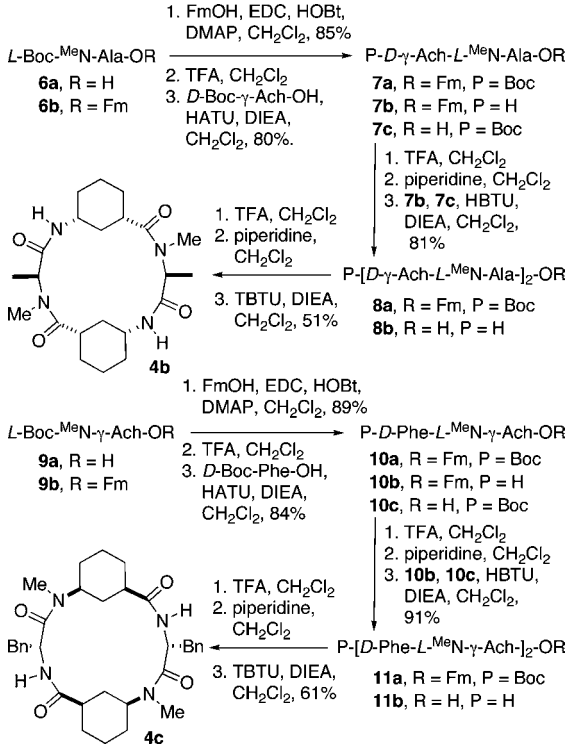
Figure 1. Top right: generic *cyclo*[(D-α-Aa-L-γ-Aca)$_n$-] peptides (α,γ-CPs) **1**−**5**. Top left: structure of a nanotube self-assembled there from by Aca-to-Aca (γ−γ) and Aa-to-Aa (α−α) hydrogen bonding between antiparallel rings. Bottom: dimers of α,γ-CPs with *N*-methylated D-α-amino acids (left) or *N*-methylated L-γ-Aca units (right), illustrating (γ−γ) and (α−α) bonding, respectively. For clarity, amino acid side chains have been omitted from the representations of the nanotube and dimers.

groups roughly perpendicular to the plane of the peptide ring, which allows β-sheetlike hydrogen bonding between rings (Figure 1). The α,γ-CPs, such as **1**−**3**, have their γ-Aca NH and C═O groups on one face (the γ-face) and their α-amino acid (α-Aa) NH and C═O groups on the other (the α-face), and because of the different HN···C═O spacings of the α- and γ-amino acids, these peptide rings can only stack through β-sheetlike hydrogen bond interactions with each other if the orientations of the rings alternate, so that α-faces bond to α-faces and γ-faces to γ-faces.[5] Thus, an SPN formed of α,γ-CPs has two alternating types of β-sheetlike sets of hydrogen bonds: one involving exclusively the NH and C═O groups of the γ-amino acid (γ−γ bonding) and the other those of the α-amino acid (α−α bonding).

The structure described above implies that, depending on the reading frame adopted, the α,γ-CP-based SPN can be regarded as composed either of dimeric repeat units that are bound internally by α−α bonding and externally by γ−γ bonding, or of dimeric repeat units that are bound internally by γ−γ bonding and externally by α−α bonding (Figure 1, top left). The thermodynamics of the formation of these nanotubes and other factors affecting the process can be studied by investigating these two kinds of repeat unit separately, to which end external hydrogen bonding between repeat units can be prevented by methylation of those of the α,γ-CP NH groups that will be outward-facing in the dimer (Figure 1, bottom).[6] Studies of dimers of this kind (which are themselves of interest as potential nanoreactors or encapsulators of guest molecules) have shown that, when the γ-Aca is *cis*-3-aminocyclohexanecarboxylic acid (γ-Ach), internally (α−α)-bound repeat units are very much more stable than internally (γ−γ)-bound units for both six amino acid α,γ-CPs **1** and eight amino acid α,γ-CPs **2**.[7] Intrigued by this, in the work described here, we investigated whether the same pattern holds for the smallest possible α,γ-CPs, tetrapeptides of type *cyclo*[(α-Aa-γ-Aca)$_2$-].

To study (γ−γ) bonding, we prepared *cyclo*[(L-^{Me}*N*-Ala-γ-Ach)$_2$-] (**4b**) by solution phase methods, as shown in Scheme 1, starting from L-Boc-^{Me}*N*-Ala-OFm (**6b**). Treatment of **6b** with TFA, followed by coupling with D-Boc-γ-Ach-OH using HATU, proceeded in high yield to give dipeptide **7a** (80%).[9] After treatment of one-half of the synthesized quantity of **7a** with 1:1 TFA/CH_2Cl_2 and the

Scheme 1. Synthesis of *cyclo*[(L-^{Me}*N*−Ala-D-γ-Ach)$_2$-] (**4b**) and *cyclo*[(D-Phe-L-^{Me}*N*-γ-Ach)$_2$-] (**4c**)



(5) The thermodynamic preference for antiparallel versus parallel β-sheet formation has been evaluated using D,L-α-peptide nanotube dimers: Kobayashi, K.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 95−98.

(6) (a) Clark, T. D.; Buriak, J. M.; Kobayashi, K.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949−8962. (b) Ghadiri, M. R.; Kobayashi, K.; Granja, J. R.; Chadha, R. K.; McRee, D. E. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 93−95.

(7) This difference can be attributed to conformational differences between ^{Me}*N*-Aca and ^{Me}*N*-Aa derivatives, to the NH group of α-amino acids being more strongly polarized than that of γ-amino acids, and possibly also to steric interactions between the *N*-methyl and carbonyl groups of the *N*-methylated α-amino acid.[8]

(8) Möhle, D.; Hofman, H. J. *J. Peptide Res.* **1998**, *51*, 19−28.

(9) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398. (b) Albericio, F.; Carpino, L. A. *Methods Enzymol.* **1997**, *289*, 104−126.

other half with 20% piperidine/CH_2Cl_2, the resulting dipeptides, **7b** and **7c**, were coupled using HBTU in the presence of DIEA, giving tetrapeptide **8a** in 81% yield. Double deprotection of **8a** with piperidine followed by TFA, and cyclization of the resulting peptide **8b**, afforded the cyclic peptide **4b** in 51% yield from **8a**.

The ^{1}H NMR spectra of **4b** in polar and nonpolar solvents (CCl_4, $CDCl_3$, MeOH, DMSO) are well defined, reflect a high degree of symmetry, and in $CDCl_3$ show a $J_{NH,H\alpha}$ coupling constant of 10.1 Hz that is typical of the all-*trans* backbone conformation required for flatness of the peptide ring. However, neither these spectra nor others run in 9:1 CCl_4/$CDCl_3$ show any signs of intermolecular hydrogen bonding, the N−H resonance of γ-Ach remaining at the same position (δ = 5.86 ppm) [regardless of concentration]. In the FT-IR spectrum,[10] amide I and amide II_{II} bands at 1621 and 1522 cm^{-1}, respectively, suggest the expected flatness of the peptide ring, but the position of the amide A band [ν(NH)], 3366 cm^{-1}, is that of an amide proton that is not involved in any hydrogen bond.

To study ($\alpha-\alpha$) bonding, we prepared *cyclo*[(D-Phe-L-^{Me}*N*-γ-Ach)$_2$-] (**4c**) using the same approach as that for **4b**. Its ^{1}H NMR spectra in polar and nonpolar solvents once again indicate a flat, all-*trans* configuration, but in this case, unlike that of **4b**, dimerization through intermolecular hydrogen bonds in nonpolar solvents is reflected by the fact that the Phe N−H signal shifts increasingly downfield as concentration is increased, from 5.9 ppm at a concentration of 1 mM to 7.7 ppm at 200 mM. This concentration dependence allowed the dimerization constant, K_a, in chloroform at 298 K to be determined as 15 M^{-1},[11] and a series of experiments carried out at different temperatures in the range of 233−313 K allowed the corresponding thermodynamic parameters $\Delta H°_{298} = -30.8$ kJ mol^{-1} and $\Delta S°_{298} = -82.0$ J K^{-1} mol^{-1} to be extracted from van't Hoff plots. The negative enthalpy and entropy, together with the observed fall in K_a with increasing solvent polarity, support the idea that the formation of dimers of **4c** is an enthalpy-driven, entropy-opposed[12] process brought about principally by intermolecular hydrogen bonding. Furthermore, the β-sheetlike nature of this bonding[10] is supported by FT-IR spectra recorded in chloroform, which not only show amide I and amide II_{II} bands (at 1627 and 1522 cm^{-1}, respectively), but also amide A bands near 3300 cm^{-1}, while a band at 3411 cm^{-1} that mainly appears in the most dilute solutions may be due to the N−H vibration of the monomer.

(10) (a) P. I. Haris, D. Chapman, *Biopolymers (Peptide Sci.)* **1995**, *37*, 251−263. (b) Krimm, S.; Bandekar, J. In *Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T., Richards, F. M., Eds.; Academic Press: Orlando, FL, 1986; pp 181−364. (c) Bandekar, J. *Biochim. Biophys. Acta* **1992**, *1120*, 123−143. (d) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 12048−12058.

(11) The chemical shift of the NH group was measured at concentrations ranging from 5.9 $\times 10^{-3}$ to 5.0 $\times$ 10^{-2} M, and the association constant was estimated using a nonlinear regression program to fit the δ_{NH} versus concentration data with the equation $\delta_{obs} = \delta_{dimer} + (\delta_{monomer} - \delta_{dimer})[(1 + 8K_aC)^{1/2} - 1]/(4K_aC)$, where δ_{obs} is the shift observed at concentration, C, and the shifts of the monomer and dimer ($\delta_{monomer}$ and δ_{dimer}) are adjusted together with the association constant K_a.

(12) (a) Searle, M. S.; Westwell, M. S.; Williams, D. H. *J. Chem. Soc., Perkin Trans. 2* **1995**, 141−151. (b) Dunitz, J. D. *Chem. Biol.* **1995**, *2*, 709−712.

Definitive evidence of the existence of dimers of **4c** in the solid state was obtained by X-ray crystallography of

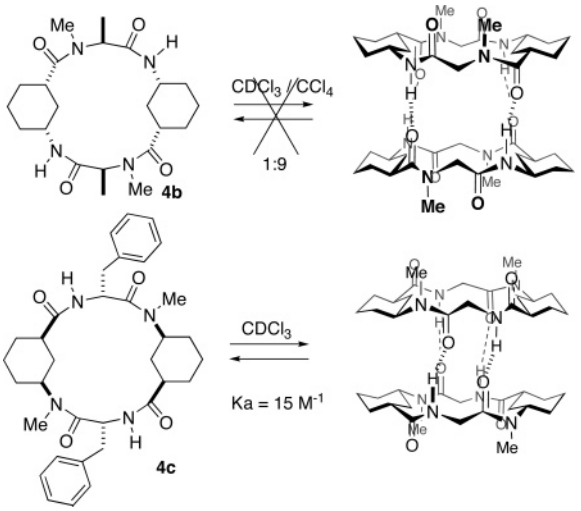


Figure 2. Compound **4c** spontaneously forms dimers in nonpolar solvents, compound **4b** does not.

colorless prismatic single crystals formed by vapor-phase equilibration of a tetrachloroethane solution of **4c** with hexane. These crystals consist of dimers in which the two CPs are stacked with antiparallel orientation and linked by a β-sheetlike set of four hydrogen bonds N···O distances of 2.91−3.08 Å (Figure 3). The fact that all the hydrogen

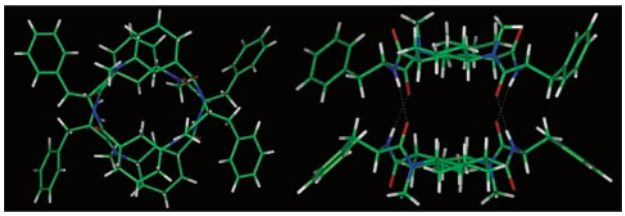
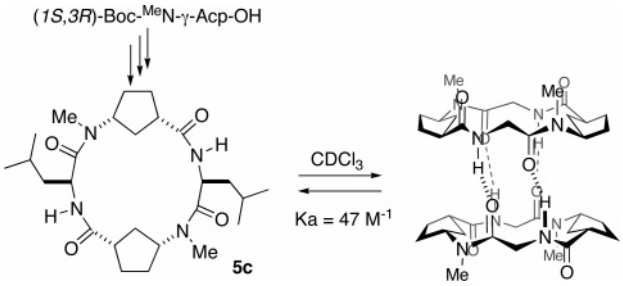


Figure 3. Crystal structure of dimeric **4c**: (a) top view; (b) side view.

bonding amide groups are slightly tilted toward the center of the dimer (Figure 3b) may explain the low enthalpic contribution to the dimerization process.

Stable ($\alpha-\alpha$)-bonded dimers were also formed when *cis*-3-aminocyclopentanecarboxylic acid (γ-Acp) was used as the γ-Aca of the α,γ-CP, instead of γ-Ach.[4d] The *cyclo*[(L-Leu-D-^{Me}*N*-γ-Acp)$_2$-] (**5c**) was prepared using the same approach as for **4b** and **4c**, but starting from D-Boc-^{Me}*N*-γ-Acp-OH (Scheme 2),[4d] and its flat, all-*trans* conformation was indicated by the characteristics of its ^{1}H NMR spectra in polar and nonpolar solvents ($J_{NH,H\gamma}$ = 9.0−9.5 Hz). As in the case of **4c**, the formation of intermolecular hydrogen bonds was indicated by the progressive downfield shift of

Scheme 2. Synthesis of **5c**, Which Spontaneously Dimerizes in Nonpolar Solvents



the α-amino N−H signal from 6.5 to 7.7 ppm as concentration was increased in nonpolar solvents. This concentration dependence showed a dimerization constant 3 times larger than that for **4c** ($K_a(CDCl_3) = 47\ M^{-1}$) and the thermodynamic parameters extracted from van't Hoff plots of $\Delta H^\circ_{298} = -12.6$ kJ mol^{-1} and $\Delta S^\circ_{298} = -10.4$ J K^{-1} mol^{-1}. Thus, dimerization of **5c** is also an enthalpy-driven, entropy-opposed process, but both enthalpic and entropic contributions are significantly smaller than those of the Ach-based tetramer (**4c**). It suggests that the Acp-based cyclic peptide is a more rigid structure hindering the optimal orientation of H-bond donors and acceptors that can be reached by the more flexible **4c**.

In conclusion, NMR, FT-IR, and X-ray diffraction data have provided conclusive evidence that α,γ-CPs with just four amino acids, like the corresponding hexa- and octapeptides, form dimers held together by β-sheetlike hydrogen bonds between their α−faces. (γ−γ)-bonded dimers, which in the case of hexa- and octapeptide α,γ-CPs are weaker than their (α−α)-bonded counterparts, seem not to be formed at all by tetrapeptide α,γ-CPs.[7,8] The (α−α)-bonded dimers are more stable than the isolated monomers by about 8.0 kJ mol^{-1}, that is, by about 2.0 kJ mol^{-1} per hydrogen bond, a value that is similar to those observed in (γ−γ)-bonded dimers of hexa- and octapeptide α,γ-CPs,[4,6] is considerably smaller than those reported for more rigid self-assembled structures based on ADAD hydrogen bond arrays,[13] and must be much smaller than the values in (α−α)-bonded dimers of hexa- and octapeptide α,γ-CPs (which have not so far been measured because dimerization is virtually complete even at low concentrations of these species).[4] It seems likely that, in tetrapeptide α,γ-CP dimers, hydrogen bonding is weaker than in the corresponding hexa- and octapeptide dimers because of conformational constraints in the small four amino acid ring.

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Supporting Information Available: Detailed descriptions of the synthesis and characterization of key compounds, and crystal data, atomic coordinates, bond lengths and angles, and anisotropic displacement coefficients of **4c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) (a) Sartorius, J.; Schneider, H.-J. *Chem.—Eur. J.* **1996**, *2*, 1446−1452. (b) Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem., Int. Ed.* **2001**, *40*, 2328−2426.