Peptide Nanostructures

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Methyl-Blocked Dimeric α,γ-Peptide Nanotube Segments: Formation of a Peptide Heterodimer through Backbone–Backbone Interactions**

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In recent years, considerable effort has been devoted to the synthesis of organic and inorganic nanotubes.[1] Among a number of other nanotube design concepts, self-assembling peptide nanotubes (SPNs)[1–6] have attracted attention for some time because of the probable ease with which they may be endowed with structural and functional properties of interest for applications in biology and materials science.[7] SPNs are formed by stacking cyclic peptides whose amino acid residues have configurations that impart the peptide ring backbone with an essentially flat shape and that orient the C=O and NH groups roughly perpendicular to the ring plane. This allows β-sheet-like hydrogen bonding between antiparallel rings (Figure 1). In particular, SPNs based on cyclic α,γ peptides (α,γ CPs) such as **1a** and **2a**, in which the requisite all-*trans* conformation is achieved by the alternation of (1*R*,3*S*)-3-aminocyclohexanecarboxylic acids (γ-Ach) with D-α-amino acids,[8] hold considerable promise for the design of nanotubes with novel structural and internal cavity properties.

The results reported herein support the possibility of constructing SPNs by using α,γ CPs of type **3**, in which the γ-amino acid is not γ-Ach but (1*R*,3*S*)-3-aminocyclopentanecarboxylic acid (γ-Acp).[9] We show that heterodimerization between γ-Ach-based and γ-Acp-based α,γ CPs is not only possible, but is favored over homodimerization for the compounds used herein. In nature, heterodimerization of peptides is common[10–12] and appears to constitute an economical means toward structural diversity and functional versatility; the same benefits may be expected to accrue from the development of a battery of α,γ CPs that are capable of forming hetero-oligomers with each other. However, whereas in all known natural cases the selectivity for hetero- rather than homodimerization is mainly due to interactions between the side chains of the component monomers, interactions of this kind are not necessary for preferential heterodimerization of the α,γ CPs studied herein. This is fortunate, because it means that the combinatorial advantages of heterodimerization are available without detriment to the possibility of exploiting side chains for other purposes.

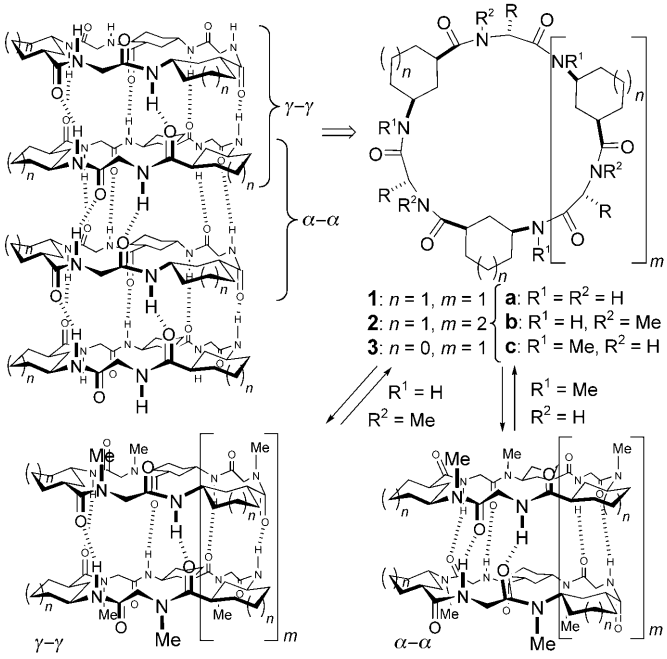

Figure 1. Design for self-assembling peptide nanotubes composed of *cyclo*[(D-Aa-(1*R*,3*S*)-γ-Aa)$_3$-] or *cyclo*[(D-Aa-(1*R*,3*S*)-γ-Aa)$_4$-] units, involving two different (α–α and γ–γ) Hydrogen-bonding patterns (Aa = amino acid). Dimer models of the two hydrogen-bonding patterns are shown at the bottom of the figure. Amino acid side chains have been omitted in the nanotube and dimer structures for clarity.

The construction of an SPN from α,γ CPs involves two different sets of β-sheet-like hydrogen bonds: one exclusively involves the NH and C=O groups of the γ-amino acid (γ–γ bonding, Figure 1) and the other, those of the α-amino acid (α–α bonding). In previous work with hexameric and octameric γ-Ach-based α,γ CPs, we found that α–α bonding was stronger than γ–γ bonding.[8] Herein, we accordingly focused on α–α bonding with the preparation of α,γ dimers in which γ–γ bonding was prevented by *N*-methylation of all the γ-amino acids as in **1c**–**3c** to avoid complications that would be irrelevant to this preliminary work.[13]

(1*R*,3*S*)-*N*-Boc-γ-Acp (**5**) was prepared from Vince's lactam (**4**)[14] by hydrogenation, acidic hydrolysis, and *N*-protection (Scheme 1) followed by successive recrystallizations from chloroform containing 0.7 equivalents of (+)-α-phenylethylamine, and was obtained with an enantiomeric purity greater than 97 %[15]. The *N*-methylated amino acid **6a** (^{Me}*N*-γ-Acp) was then obtained by treatment of **5** with sodium hydride and iodomethane in THF, and its fluorenyl-protected ester **6b** by reaction of **6a** with fluorenylmethanol.

The α,γ CP *cyclo*[(D-Leu-(1*R*,3*S*)-^{Me}*N*-γ-Acp)$_3$-] (**7a**, Figure 2) was synthesized by standard procedures and characterized by NMR spectroscopy and mass spectrometry (see Supporting Information). Its ^{1}H NMR spectra in polar and nonpolar solvents (CCl_4, $CDCl_3$, MeOH, DMSO) are well-defined, reflect a high degree of symmetry, and show a $J_{NH,H\alpha}$

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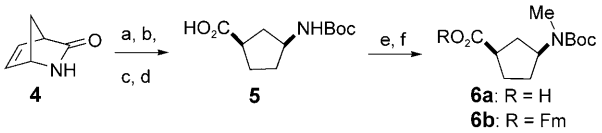

Scheme 1. Synthesis of (1*R*,3*S*)-Boc-^{Me}*N*-γ-Acp-OH (**6a**) and (1*R*,3*S*)-Boc-^{Me}*N*-γ-Acp-OFm (**6b**) from Vince's lactam (**4**); a) H_2, Pd/C, MeOH, 99%; b) HCl (10%), 90%; c) $(Boc)_2O$, *N*,*N*-diisopropylethylamine, H_2O/dioxane, 89%; d) resolution: (+)-α-phenylethylamine, $CHCl_3$/hexanes: $[\alpha]_D = -16.8$ ($c = 1.0$ in MeOH), >97% *ee*; e) NaH, MeI, THF, 98%; f) FmOH, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, 1-hydroxybenzotriazole, 4-(dimethylamino)pyridine, CH_2Cl_2, 92%. Boc = *tert*-butoxycarbonyl, Fm = fluorenylmethyl.

coupling constant of 9.4 Hz, which is typical of the all-*trans* backbone conformation required for the flatness of the peptide ring (which, in turn, favors the formation of hydrogen-bonded assemblies such as the SPN shown in Figure 1). In nonpolar solvents, the formation of a dimer ($\mathbf{8}_{7a}$) is reflected by the downfield shift of the NH signal of Leu. The fact that the location of this signal ($\delta = 8.23$ ppm) remains constant at concentrations as low as 1×10^{-4} M, regardless of methanol composing up to 30% of the solvent or heating at 323 K, shows that the association constant for dimer formation must be at least $10^5\,M^{-1}$. FTIR spectra recorded in chloroform display bands at 1626, 1534, and 3304 cm^{-1} which, like analogous bands observed for other all-*trans* cyclic peptides linked by hydrogen bonds in dimers or SPNs,[2,8,13] resemble the amide I, amide II_{II}, and amide A bands typical of protein β sheets.

X-ray crystallography of a colorless prismatic crystal obtained from a solution of peptide **7a** in chloroform by vapor-phase equilibration with hexane confirmed the α–α dimerization of essentially flat, antiparallel rings by means of a β-sheet-like array of six hydrogen bonds with interatomic N···O distances of 2.88–2.98 Å (Figure 3a,b). The drum-shaped dimer has an approximate van der Waals internal diameter of 5.4 Å, and its cavity is occupied by a partially disordered chloroform molecule. The body-centered struc-

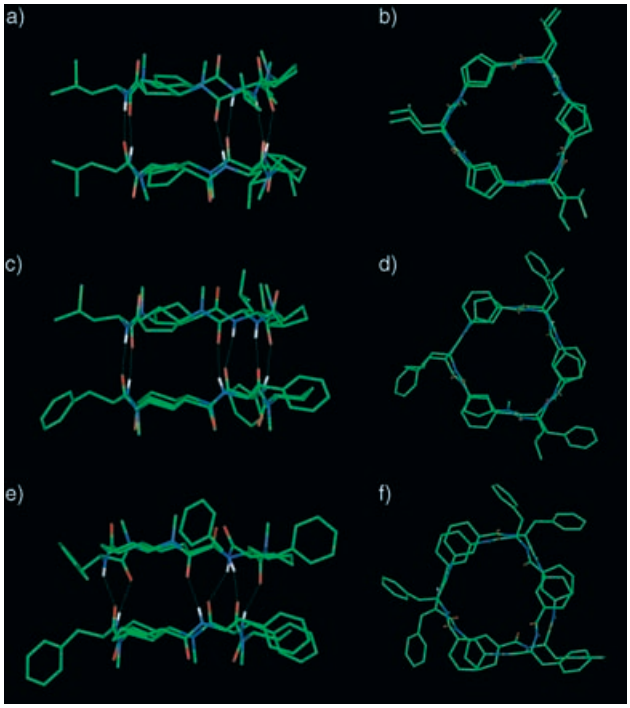

Figure 3. a) Views from the sides (a, c, e) and down the axes (b, d, f) of a,b) homodimers $\mathbf{8}_{7a}$ and e,f) **10**[8a], and of c,d) heterodimer $\mathbf{11}_{7a-9}$ in the solid state; chloroform molecules in the central cavities have been removed for clarity.

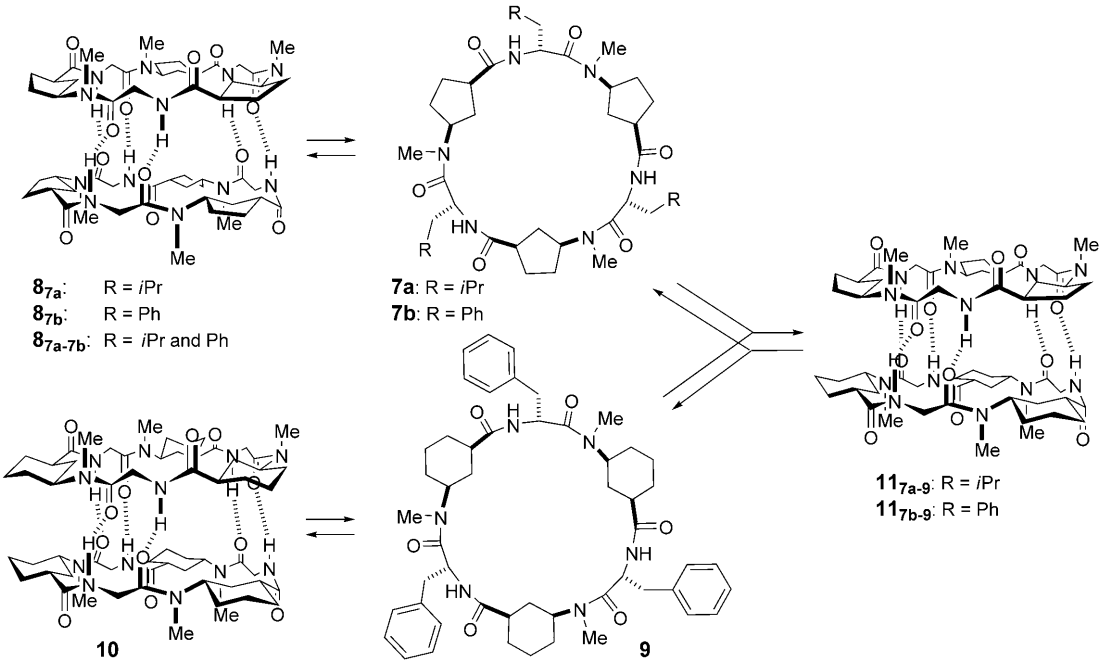

Figure 2. Homo- and heterodimerization of *cyclo*[(D-Leu-(1*R*,3*S*)-^{Me}*N*-γ-Acp)$_3$-] (**7a**), *cyclo*[(D-Phe-(1*R*,3*S*)-^{Me}*N*-γ-Acp)$_3$-] (**7b**) and *cyclo*[(D-Phe-(1*R*,3*S*)-^{Me}*N*-γ-Ach)$_3$-] (**9**).

ture of the crystal forms channels by stacking the dimers along the crystallographic *a* axis, each dimer making van der Waals contacts with the next through its γ face carbonyl and *N*-methyl groups.

The γ-Ach-based **7a** analogue *cyclo*[(D-Phe-(1*R*,3*S*)-^{Me}*N*-γ-Ach)$_3$-] (**9**) also forms stable homodimers **10** in nonpolar solvents, with an association constant estimated to be at least $10^5\,M^{-1}$, as in the case of **7a** (Figure 2).[8] The possibility of heterodimer formation was confirmed upon the addition of 0.8 equivalents of **7a** to a solution of **9** (2.1 mM) in chloroform, which resulted in the appearance of a new species with a ^{1}H NMR spectrum that did not correspond to either of the possible homodimers. The dimeric nature of the new species, $\mathbf{11}_{\mathbf{7a\text{-}9}}$, was shown by NOE cross-peaks between the signals of $H\gamma_{Acp}$ (at $\delta = 4.92$ ppm) and $H\alpha_{Ach}$ (at $\delta = 2.62$ ppm). Definitive evidence of heterodimerization in the solid state was obtained by X-ray crystallography of the colorless prismatic single crystals recovered from a 1:1 solution of **7a** and **9** in chloroform. As expected, the monomeric cyclic peptides form heterodimers in which the two essentially flat rings are antiparallel, with their α faces linked in β-sheet fashion through six hydrogen bonds with N···O interatomic distances of 2.82–2.90 Å (Figure 3). The dimers stack closely along the *a* axis, each making van der Waals contacts with its neighbor with γ-face carbonyl and *N*-methyl groups. The resulting channel contains two chloroform molecules per unit, one in the cavity of the heterodimer and a second at variable sites between successive dimers.

Notably, the heterodimer $\mathbf{11}_{\mathbf{7a–9}}$ was in fact about 30-fold more abundant than the homodimer if prepared as described above from a mixture of **7a** and $\mathbf{9}$,$\mathbf{8}_{\mathbf{7a}}$ (0.8:1) which at 298 K corresponds to an energy difference of 2.0 kcal mol^{-1}. To determine whether this selectivity was derived from interactions between the side chains of the monomers, we investigated the dimerization of **7a** with *cyclo*[(D-Phe-(1*R*,3*S*)-^{Me}*N*-γ-Acp)$_3$-] (**7b**).[16] If the stability of $\mathbf{11}_{\mathbf{7a–9}}$ were the result of side-chain–side-chain interactions, the potential heterodimer $\mathbf{8}_{\mathbf{7a–7b}}$, in which the side chains are the same as those of $\mathbf{11}_{\mathbf{7a–9}}$ yet not the two backbones, is expected to be at least as stable as is $\mathbf{11}_{\mathbf{7a–9}}$ relative to $\mathbf{8}_{\mathbf{7a}}$. Nevertheless, the ^{1}H NMR spectrum of a 1:1 mixture of **7a** and **7b** in chloroform showed the presence of dimers $\mathbf{8}_{\mathbf{7a}}$, $\mathbf{8}_{\mathbf{7b}}$, and $\mathbf{8}_{\mathbf{7a–7b}}$ in almost equal concentrations, which implies that all three species have similar stabilities. This rules out the possibility of significant interactions between the side chains of opposing rings. Consistent with this conclusion, the addition of 0.9 equivalents of **9** to a solution of **7b** (4.6 mM) in chloroform resulted in the formation of heterodimer $\mathbf{11}_{\mathbf{7b–9}}$ without any observable trace of the homodimer **10**; this shows an even greater difference in stability between hetero- and homodimers than in the case of $\mathbf{11}_{\mathbf{7a–9}}$ and **10**.

The cause of the higher stability of the heterodimers is unclear. However, inspection of the crystal structures of $\mathbf{8}_{\mathbf{7a}}$, **10**[8a] and $\mathbf{11}_{\mathbf{7a–9}}$ (Figure 3) suggests that the heterodimer is more stable through an improved alignment of hydrogen-bond donors and acceptors. This could be due in part to more favorable packing between cyclopentyl and cyclohexyl than cyclopentyl–cyclopentyl or cyclohexyl–cyclohexyl ring packing. This hypothesis is supported by the intradimer distances between CPs in the crystals (see Supporting Information) and by those calculated from the N–H stretch frequencies ($\tilde{\nu} =$ 3304, 3309, and 3300 cm^{-1} in $\mathbf{8}_{\mathbf{7a}}$, **10** and $\mathbf{11}_{\mathbf{7a–9}}$, respectively; see Supporting Information). However, further studies might clarify this issue.

In conclusion, NMR and FTIR spectroscopy and X-ray diffraction data show conclusively that cyclic α,γ hexapeptides containing three γ-Acp units can form stable cylindrical dimers in which antiparallel peptide rings are linked by a β-sheet-like set of six hydrogen bonds. Even greater stability is shown by the heterodimers of these γ-Acp α,γ CPs with γ-Ach α,γ CPs. Potential applications for nanotubes developed from appropriately functionalized α,γ CPs include their use as logic gates, biosensors, catalysts, molecular receptors, and molecule containers.

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[16] In the absence of other peptides, **7b** formed highly stable homodimers ($\mathbf{8}_{\mathbf{7b}}$) in chloroform and other nonpolar solvents.

