Self-Assembling Macrocycles

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Control of Duplex Formation and Columnar Self-Assembly with Heterogeneous Amide/Urea Macrocycles**

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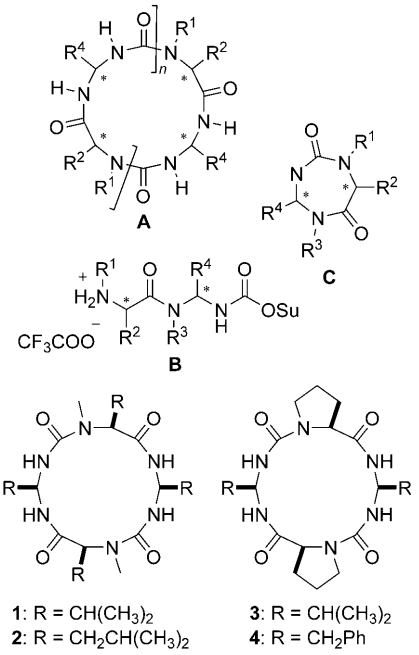
Owing to their diversity in size and shape, easy access, and biocompatibility, peptides are versatile units for the construction of H-bonded tubular assemblies and other biomimetic materials with potentially useful applications.[1] Peptide nanotubes (PNTs) have been obtained through multiple and complementary approaches,[1,2] including the formation of hollow β helices,[3] barrel–hoop motifs from stacked macrocyclic peptides,[4] barrel–stave motifs from rigid-rod peptide conjugates,[5] helical pores from cationic, zwitterionic,[6] and dendritic dipeptides,[7] and large tubes from linear and cyclic amphiphilic peptides.[8]

Originally designed from α-peptides made of D- and L-amino acids,[4,9] the range of flat macrocyclic systems forming cylindrical β-sheet-like assemblies has been expanded to include oligoamides formed of higher amino acid homologues (e.g. β- and δ-peptides)[10] and peptide hybrids (e.g. α,β-,[11a] α,γ-,[11b–f] and α,ε-peptides[11g]). Tubular sheet-like assemblies are however not restricted to oligoamides. The urea group for example, which shares a number of features with the amide linkage, namely rigidity, planarity, polarity, and hydrogen bonding capacity, is an interesting surrogate. Macrocyclic biotic and abiotic *N*,*N*′-linked oligoureas have a unique propensity to self-organize into polar H-bonded nanotubes.[12–14]

Partial peptide backbone *N*-methylation has been introduced as a general strategy to generate truncated stacks (i.e., H-bonded dimers), which are useful in gaining access to the thermodynamics of nanotube formation.[9c,d,11b–e] Herein, we describe biotic macrocyclic amide/urea hybrids with partially *N*-alkylated backbones **A** as new candidates for the formation of H-bonded dimers. In these systems, we show that 1) backbone *N*-alkylation does not necessarily compromise extended columnar and tubular self-assembly, 2) parallel versus antiparallel stacking can be controlled by the degree of backbone rigidification, and 3) structural water molecules may function as bridging units to direct tubular and columnar growth.



Our approach to C_n ($n=1$ to 5) hybrid amide/urea macrocycles of type **A** is based on cyclooligomerization of the dipeptide-derived precursor, $^{+}$H-Xaa-*g*Xbb-COOSu (**B**).[15] Small-ring formation leading to the 1,3,5-triazepan-2,6-dione dipeptidomimetic skeleton **C**[15a–c] readily occurs when the *cis*-conformation around the amide bond in **B** is populated ($R^3 \neq H$). To promote cyclooligomerization, we thus focused on starting dipeptide sequences featuring a secondary amide bond, and *N*-alkylated on Xaa ($R^3 = H$, $R^1 \neq H$).[16] A series of four enantiopure 14-membered C_2 symmetric macrocycles (**1**–**4**) have been prepared starting from homochiral *N*MeVal-Val, *N*MeLeu-Leu, Pro-Val, and Pro-Phe dipeptide sequences, respectively.

Single crystals of **1** and **2** suitable for X-ray crystallographic analysis were grown by slow evaporation of a solution of acetonitrile and methanol. The crystal structures were solved in the *P*1 and $I4_1$ space groups,[16] respectively. In both structures (Figure 1 a, b), the main chain adopts a rectangular shape with sides of length 3.8 Å × 4.8 Å.

The amide and urea groups are perpendicular to the mean plane of the ring, and their carbonyl groups point in opposite directions. Whereas ϕ angles of L-amino acid residues in **1** and **2** adopt standard negative values (ca. −97°), *gem*-diamino

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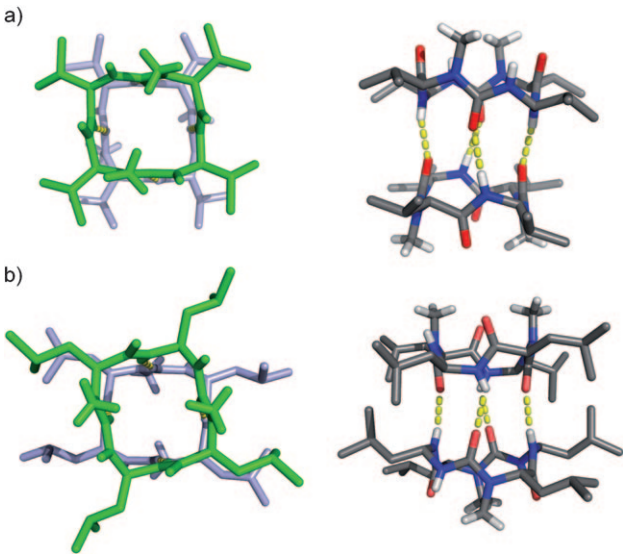

Figure 1. Representations of cylindrical H-bonded dimers of **1** (a) and **2** (b). Left: individual molecules shown in gray and green. Right: C gray, N blue, O red, H white, H-bonds yellow. Hydrogen atoms of side chains are omitted for clarity.

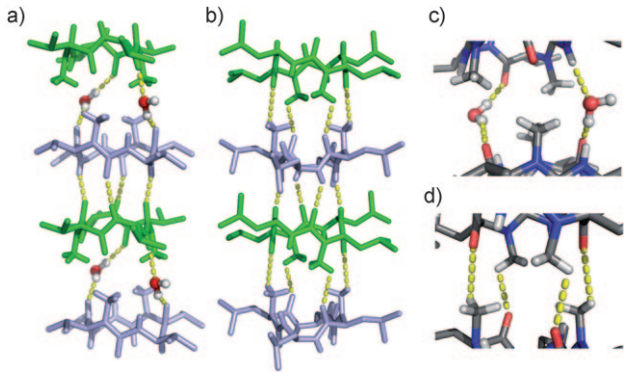

Figure 2. Formation of columnar stacks by axial packing of H-bonded dimers in **1** (a) and **2** (b); individual molecules are shown in gray and green. c),d) Close-ups of the interfaces between duplex in **1** (c) and **2** (d) illustrating the different modes of column stabilization. C gray, N blue, O red, H white, H-bonds yellow. H atoms of side chains are omitted for clarity.

alkyl residues are characterized by positive pseudo ϕ angle values (ca. $+60°$), which are generally accessible to the D-amino acid residues. As a result, the two NH groups of *gem*-diamino alkyl residues point in opposite directions. By analogy to partially *N*-methylated D,L-α-peptides and α,γ-peptides,[9c,d,11b–e] the cyclic urea/amide oligomers **1** and **2** self-assemble in an antiparallel manner to form H-bonded dimers maintained by a set of four strong H-bonds between urea carbonyl groups and amide NH groups. N···O distances range from 2.79–2.94 Å for **1** and 2.88–2.90 Å for **2**. ^{1}H NMR studies of **1** and **2** suggest that the geometry of the ring observed in the solid state is essentially retained in solution.[16] The formation of a sheet-like arrangement in solution was inferred from FTIR spectrum of **2** recorded in $CHCl_3$ (20 mM) which shows amide A, I, and II bands at 3356, 1642, and 1521 cm^{-1} similar to previously reported peptide and oligourea nanotubes.[16,17] Concentration-dependent NMR experiments provided further evidence for the formation of H-bonded dimers in solution (in $CDCl_3$ or CD_2Cl_2 stored over 4 Å molecular sieves). In agreement with the proposed antiparallel dimerization, proton resonances of amide NH but not urea NH groups experienced a significant downfield shift ($\delta = 7.43$ to 8.03 ppm at 223 K for **2**) as the concentration increased from 0.1 mM to 109 mM. The presence of a unique set of signals suggested fast equilibrium between monomer and dimer on the NMR timescale.[16] Fitting urea NH chemical shifts by a dimerization isotherm gave K_{dim} (CD_2Cl_2) of 41 M^{-1} at 223 K.[18] Van't Hoff plot analysis over the range 213–253 K afforded values of -34.8 kJ mol^{-1} and -136.3 J K^{-1} mol^{-1} for the enthalpy and entropy of dimerization of **2** in CD_2Cl_2, respectively.

Interestingly, both dimers of **1** and **2** form columnar stacks parallel to the *b* axis and *c* axis, respectively, with a mean distance between H-bonded dimers of ca 4.5 Å (Figure 2). Column stabilization in **2** is essentially driven by weak C–H···O=C bonds (C···O 3.11 Å, H···O 2.61 Å, C-H-O 111.3°) between methyl groups and amide carbonyl of two H-bonded dimers and by van der Waals interactions between interdigitated isobutyl side chains of adjacent columns (Figure 2b,d). A totally different mode of column stabilization is observed in the structure of **1**, in which two H-bonded dimers are interconnected by two bridging water molecules (W_1 and W_2) that are an integral part of the tube architecture (Figure 2a,c). Indeed, W_1 and W_2 lie on the edge of the tube approximately in the alignment of the *g*Val $^{\alpha}$C atoms and equidistant from two dimers. However, the two molecules differ in their bridging mode. W_1 acts as a donor and bridges two amide carbonyl groups, whereas W_2 acts as both an acceptor and a donor to bridge urea NH and amide carbonyl groups.

Substituting proline for the *N*-methyl amino acids in urea/amide cyclodimers has dramatic consequences on both ring geometry and self-assembly properties of resulting cyclodimers. Compound **3** crystallized from $CHCl_3$ in the $P2_12_12$ space group. Crystals of **4**, obtained by slow evaporation of a mixture of acetonitrile and water, formed in the space group $I4_1$.[16] Both **3** and **4** have a very similar rectangular shape with sides of length 3.8 Å × 4.8 Å (Figure 3). In contrast to **1** and **2**, *gem*-diaminoalkyl residues in **3** and **4** feature negative pseudo ϕ angle values, and as a result, amide and urea NH groups are now pointing on one side of the ring, the carbonyl groups being sequestered on the other side (Figure 3a). This novel ring geometry in **3** and **4** leads to the formation of a novel type of columnar arrangement in the crystal. Although backbone–backbone H-bonding is not observed, rings are linked in a parallel orientation by bridging water molecules (Figure 3b–d). Each water molecule is tightly sandwiched between two neighboring rings with the following H-bonding regime: The water oxygen atom is doubly hydrogen-bonded to the amide protons on one ring (N···O_w distance: 2.99 in **4** and 3.08 Å in **3**) and to urea carbonyls of a second unit (O···O_w distance: 2.68 in **4** and 2.73 Å in **3**). In this regime, the urea carbonyl groups and amide protons point more toward the water oxygen than along the column axis. The columns of *cyclo*(Pro-*g*Phe-CO)$_2$

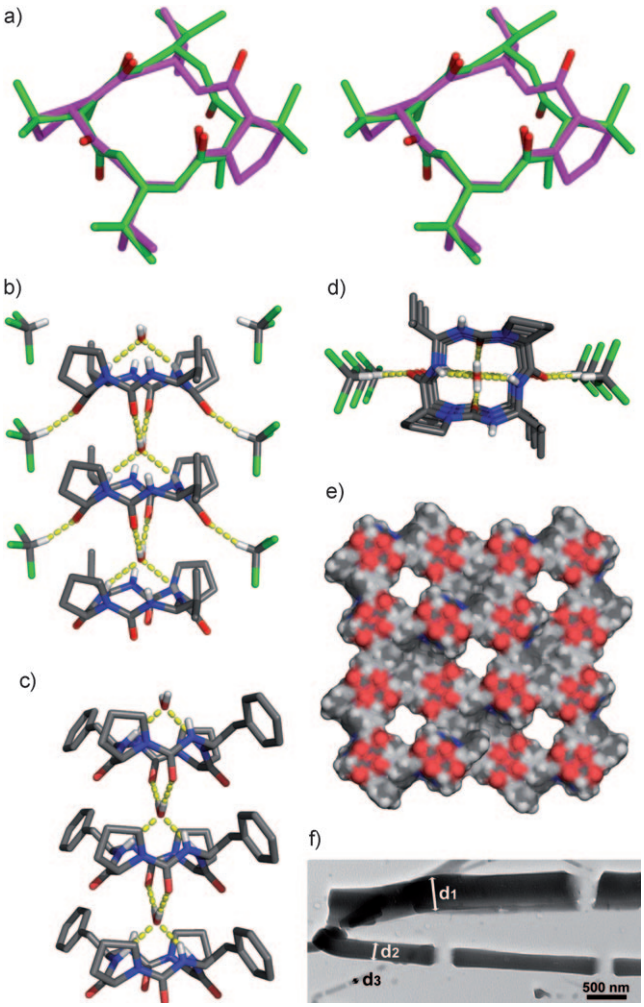

Figure 3. a) Overlay of **1** and **3**. b),c) Representations of the crystal structures of **3** (b) and **4** (c) showing the bridging water molecules and their binding mode to upper and lower rings. C gray, N blue, O red, H white, H-bonds yellow. d) H-bonded columns of **3** viewed along the *c* axis. e) Channels formed by lateral packing of columns in **4** viewed along the channel axis. f) TEM image of tubular nanostructures of **4**. External diameters of the tubes: $d1 = 200$ nm, $d2 = 90$ nm, $d3 = 20$ nm.

(**4**) pack in a square array to give channels of about 5.2 Å average cross-section that are centered on the *c* axis (Figure 3e). Of note, TEM imaging revealed another level of hierarchical organization, with the formation of well-defined tubular nanostructures of 20 nm to 1.2 μm diameter (Figure 3f and Supporting Information, Figure S5).[19]

Herein, cyclooligomerization of chiral dipeptide-derived building blocks was used to generate hybrid urea/amide macrocycles. The possibility of generating heterogeneous backbones spectacularly expands the structure space attainable with a relatively small pool of residue types. A high level of hierarchical and directional control has been achieved in these systems. Recent findings suggest that mixing amide and urea linkages can be used to create molecules with improved properties compared to cognate oligoamides (e.g. antibacterial foldamers,[20] receptors for anions with high selectivity for oxyanions[21]). Studies of the anion binding properties of hybrids **1**–**4** and of larger ring systems will be part of future development of this work.

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[16] See the Supporting Information.

[17] Dimer formation was also supported by ESI-MS (Ref. [16]).

[18] K_{dim} ($CDCl_3$) and K_{dim} (CD_2Cl_2, 4 Å MS) at 243 K were found to be very similar (8 and 7 M^{-1}, respectively), thus suggesting that the presence of a small amount of water may have only a limited influence on dimer formation.

[19] The aggregation behavior of **3** and **4** was also investigated in apolar solvent by ^1H and diffusion-ordered spectroscopy (DOSY) (Ref. [16]).

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