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Simultaneous Parallel and Antiparallel Self-Assembly in a Triazole/Amide Macrocycle Conformationally Homologous to D-,L-α-Amino Acid Based Cyclic Peptides: NMR and Molecular Modeling Study

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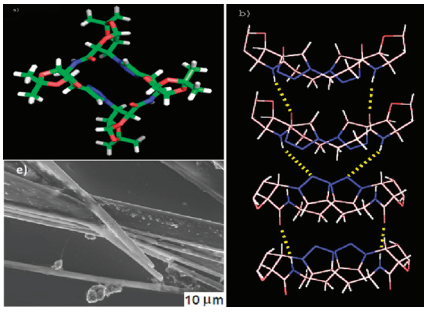
ABSTRACT



A 1,4-linked triazole/amide based peptidomimetic macrocycle, synthesized from a triazole amide oligomer of *cis*-furanoid sugar triazole amino acids, possesses a conformation resembling the D-,L-α-amino acid based cyclic peptides despite having uniform backbone chirality. It undergoes a unique mode of self-assembly through an antiparallel backbone to backbone intermolecular H-bonding involving amide NH and triazole N2/N3 as well as parallel stacking via amide NH and carbonyl oxygen H-bonding, leading to the formation of a tubular nanostructure.

In recent years, the construction of H-bonded organic tubular assemblies of macrocyclic peptides has become an important area of research for biomimetic materials with potentially useful applications.[1] Cyclic D,L-α-peptides,[2,3] cyclic β- and δ-peptides,[4] peptide hybrids (e.g., α,β-,[5a] α,γ-,[5b] and α,ε-peptides[5c]), and oligoureas[6] constitute the range of all known flat macrocyclic molecules forming β-sheet-like assemblies. However, β-peptide based novel peptidomimetics represent a significant modification of the α-peptide analogues toward biomedical

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(1) (a) Gao, X.; Matsui, H. *Adv. Mater.* **2005**, *17*, 2037. (b) Ulijn, R. V.; Smith, A. M. *Chem. Soc. Rev.* **2008**, *37*, 664.
(2) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem.* **2001**, *113*, 1016. *Angew. Chem., Int. Ed.* **2001**, *40*, 988.
(3) (a) Clark, T. D; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651. (b) Ghadiri, M. R.; Kobayashi, K.; Granja, J. R.; Chadha, R. K. *Angew. Chem.* **1995**, *107*, 76. (c) Clark, T. D.; Kobayashi, J. M.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949.
(4) (a) Seebach, D.; Matthews, J. L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L. B. *Helv. Chim. Acta* **1997**, *80*, 173. (b) Gauthier, D.; Baillargeon, P.; Drouin, M.; Dory, Y. L. *Angew. Chem.* **2001**, *113*, 4771. (c) Baillargeon, P.; Bernard, S; Gauthier, D.; Skouta, R.; Dory, Y. L. *Chem.—Eur. J.* **2007**, *13*, 9223.
(5) (a) Karle, I.; Handa, B. K.; Hassall, C. H. *Acta Crystallogr., Sect. B* **1975**, *31*, 555. (b) Amorin, M.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 2844. (c) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 9372 and references therein.
(6) (a)Fischer, L.; Decossas, M.; Briand, Jean-Paul.; Didierjean, C.; Guichard., G. *Angew. Chem., Int. Ed.* **2009**, *48*, 1625 and references therein.

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applications because they show higher stability in vivo and do not bind to the active site of proteolytic enzymes. We were particularly attracted to heterocyclic backbone modified macrocyclic sugar based β-peptides because they possess greater conformational flexibility to form flat ring structures, an important condition for intermolecular H-bond directed self-assembly, and also because the peptide main chain can provide an opportunity to alter and fine-tune the chemical properties of peptide nanotubes.

Synthesis of cyclo-tri-β-peptides having a 14-helix architecture from the amide oligomer of a *cis*-furanoid sugar amino acid[7] encouraged us to synthesize a 1,4-linked triazole backbone containing a hybrid triazol/amide macrocycle from triazole-amide oligomers of *cis*-furanoid sugar triazole amino acids basically for two reasons: (a) the 1,4-triazole linkage serves as a trans peptide bond isostere in terms of planarity, polarity, and hydrogen bond donating as well as accepting capacities;[8a−c] (b) separation of the triazole and amide by a two carbon unit permits it to adopt a chair-like conformation effectively so that the triazole ring can also participate in noncovalent interaction during the nanotube formation. Our study highlights that replacing the amide bond in a cyclo-β-peptide with a 1,4-triazole linkage leads to a conformation resembling the D-,L-α-amino acid based cyclic peptides[5b] in the orientation of the amide groups but the mode of self-assembly is distinctly different. Both parallel and antiparallel β-sheet intermolecular hydrogen bondings exist in the triazole/amide macrocycle with identical chirality throughout the backbone unlike the alternatingly chiral cyclic D-,L-α-peptides (only antiparallel β-sheet intermolecular hydrogen bonding).

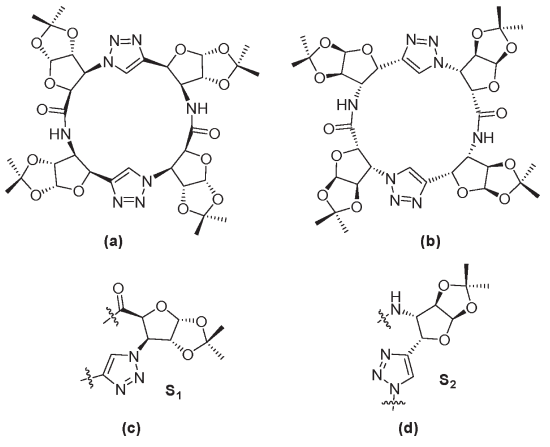
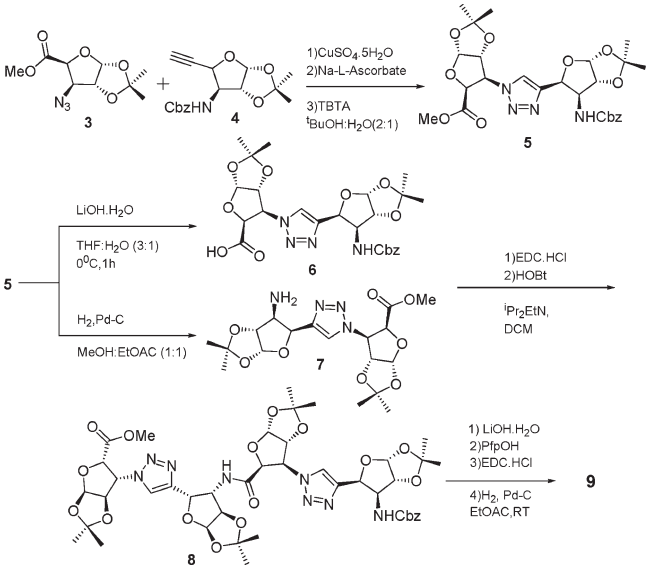


Figure 1. (a and b) Two rotamers of the pseudo cyclic β-peptide **9**; (c and d) two sugar derived components of that peptide.

The basic intermediate for the solution phase synthesis[9] of the peptide macrocycle **9** is the 1,2,3-triazole di-β-peptide isostere **5**, readily synthesized from N-Cbz protected *cis*-furanoid homopropargyl sugar amine **4**[9,10] from protected sugar amine **2** and *cis*-furanoid azido ester **3**[7a] derived from diacetone glucose **1** via Cu(I) catalyzed azide alkyne cycloaddition.[11a,b] The intermediate dimeric Cbz protected triazole amino ester was converted to two different intermediates: the free amino ester **7** by hydrogenation and the Cbz protected amino acid **6** by LiOH·H_2O treatment. After coupling the two intermediates by standard protocol (using EDC·HCl and HOBt), the ester group was hydrolyzed again by LiOH·H_2O to obtain the tetramer Cbz protected amino acid **8**. Activation of the linear tetramer acid **8** by pentafluorophenol and subsequent hydrogenation via in situ cyclization gave the final triazole/amide macrocycle compound **9** which was purified by preparative HPLC (Scheme 1).

Scheme 1. Preparation of Compound **9**



^{1}H NMR spectra of **9** in polar and nonpolar solvents (DMSO, CCl_4−$CDCl_3$, MeOH) are well-defined, reflecting a high degree of C_2 symmetry. The observed coupling constant $J_{NH,C\beta H(S2)}$ is approximately 3.6 Hz, implying a pseudo positive φ angle (i.e., ∼+60), which is generally accessible with the D-α-amino acid residues;[6] the value observed in typical cyclo-β-peptides is nearly 8.5 Hz.[7a,b] The low intensity of ROE cross peaks of the triazole ring proton with the C(β) proton of sugar S1 and C(α) proton of sugar S2 (Figure 1) definitely indicates a pseudo trans configuration, which is normally observed with L-α-amino

(7) (a) Jagannadh, B.; Reddy, M. S.; Lohitha Rao, C.; Prabhakar, A.; Jagadeesh, B.; Chandrasekhar, S. *Chem. Commun.* **2006**, 4847. (b) Fujimura, F.; Fukuda, M.; Sugiyama, J.; Morita, T.; Kimura, S. *Org. Biomol. Chem.* **2006**, *4*, 1896.

(8) (a) Angell, Yu L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674. (b) Angelo, N.; Arora, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 17134. (c) Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *ChemBioChem* **2005**, *6*, 1167.

(9) See Supporting Information.

(10) (a) Hauske, J. R.; Dorff, P.; Julin, S.; Martinelli, G.; Bussolari, J. *Tetrahedron Lett.* **1992**, *33*, 3715. (b) Ohira, S. *Synth. Commun.* **1989**, *19*, 561.

(11) (a) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596. (b) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.

acid residues,[8a−c] suggesting that the peptide backbone conformation is similar to that of cyclic peptides composed of alternative D-,L-α-amino acids (Figure 2). Furthermore, dipole−dipole interaction between furanoid C−O and an adjacent triazole ring of one pseudo cyclic peptide backbone unit can also provide an additional explanation for this conformation (see Supporting Information).[8b] Molecular modeling and ROESY spectroscopic studies reveal that the cyclic peptide structure tends to minimize nonbonded intramolecular side chain−side chain and side chain−backbone interactions by adopting a flat ring-shaped conformation. The amide and triazole backbone was perpendicular to the mean plane of the peptide ring where N2−N3 (equivalent to a carbonyl group[8a−c]) and amide carbonyl, as well as amide NH and triazole CH (equivalent to NH[8a−c]), groups are alternatively oriented along opposite faces of the peptide backbone.

The FT-IR spectrum for compound **9** recorded in $CHCl_3$ solution, which showed the amide A, I, and II bands at 3304, 1677, and 1537 cm^{-1}, suggested the formation

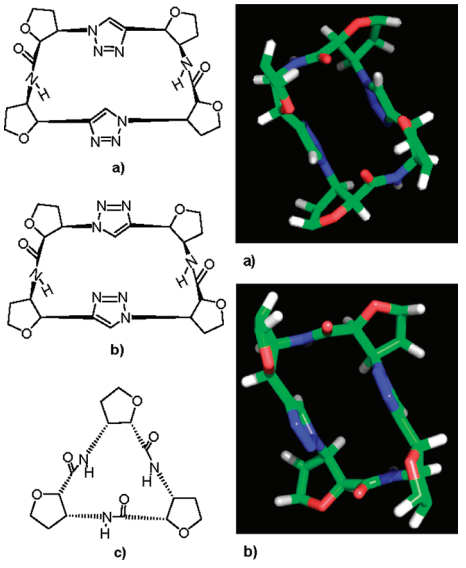


Figure 2. Two possible lowest energy conformations of pseudo peptidomimetic triazole/amide macrocycle **9**: (a) structure resembling the D-,L-amino acid based cyclic peptides, (b) structure resembling typical cyclo-β-peptides, (c) structure of typical cyclo-β-peptides[7a] containing *cis*-furanoid sugar amino acids. ROESY and molecular modeling (modeling using biopolymer module, minimization using CVFF force field on Insight II Silicon Graphics O_2 workstation 98.0 via in vacuo simulation) study shows that structure (a) is energetically more stable (by about 16.9 kcal/mol) than (b). For clarity isopropylidene ring residues are not shown.

of a β-sheet-like structure as previously reported for cyclic peptides.[8c,9] Investigation of concentration dependent ^1H NMR at −20 °C in a nonpolar solvent such as $CDCl_3/CCl_4$ (2:3) also provided evidence for the formation of intermolecular hydrogen bonding between the two peptide rings as the NH chemical shift changed from 8.93 to 8.80 on dilution. Additionally it also provided information on dimeric β-structure formation as a substantial downfield shift (> 0.1 ppm) was noted for signals of a Cα proton of sugar S_1 in going from a polar (DMSO) to nonpolar solvent (2:3 $CDCl_3/CCl_4$) (see Supporting Information). The presence of a unique set of signals suggested fast equilibrium between the monomer and dimer (or higher order) on the NMR time scale.[6]

ESI-MS of the cyclic peptide **9** recorded in $CDCl_3/CCl_4$ (2:3) showed the pseudo molecular ion peaks of the monomeric species at 811 (M+Na)+ and the dimeric species at 1599 (2M+Na)+. The molecular association and self-assembly of the cyclic peptide **9** into nanorods, whose diameter was in the range of 120−400 nm and length several micrometers, was evident from SEM and TEM images obtained in $CDCl_3/CCl_4$.[9]

In analogy with the cyclic D-,L-α-peptides, the cyclic triazole/amide oligomer can self-assemble in two modes of stacking: (a) basically antiparallel (L–L) and (b) parallel (L–D). Four strong H-bonds between amide NH and N2−N3 atoms of the triazole ring lead to the formation of the (L–L) mode of stacking (Figure 3). In the parallel (L–D) mode of stacking two H-bonds form between amide NH and amide carbonyl groups and two between C−H of a triazole ring and N2−N3 atoms of another half.

The ROESY spectrum in $CDCl_3$ provides evidence for interaction between S1(α)−S1(δ) and S2(α)−S2(δ) hydrogen atoms of the sugar moiety which can only occur from parallel mode of stacking (L–D) as reported previously for furanoid sugar amino acid based cyclic β-peptides (Figure 4).[7a] Interestingly, in a more nonpolar solvent such as (2:3) $CDCl_3/CCl_4$ not only did the chemical shifts change (> 0.1 ppm for S1(α) and S1(β) protons) but also an additional interaction between S1(α) and S2(β) hydrogen atoms of the furanoid sugar moiety was observed; evidence for the parallel interaction was however retained. This observation leads to the conclusion that an antiparallel mode of stacking (L–L) is maintained by four strong H-bonds between NH and N2−N3 atoms of the triazole ring.

Literature reports on molecular modeling and other studies on cyclic D-,L-α-peptides and also α,γ-peptides show that the α-peptides always favor antiparallel β-sheet hydrogen bonding over the parallel one where steric interaction occurs between the side chain and backbone. In our system with identical backbone chirality, the steric interaction is minimal[9] for both antiparallel and parallel arrangements. Therefore there is a possibility for both types of β-sheet hydrogen bonding to exist.

To establish whether the two types of hydrogen bondings exist in different tubular sheets or are mixed up in one tubular sheet structure, we carried out a low temperature NMR study where the unique set of signals was retained. From this result we conclude that two β-sheet hydrogen bonding arrangements exist in one tubular sheet because the existence of two tubular sheets with different modes of

(12) (a) Adhikary, N. D.; Chattopadhyay, P. *Eur. J. Org. Chem.* **2010**, 1754. (b) Majhi, T. P.; Neogi, A.; Mukhopadhyay, R.; Chattopadhyay, P. *Synthesis* **2005**, 2307.

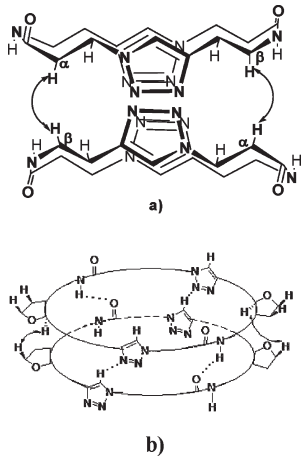


Figure 3. Schematic representation of solution self-assembly by 2-fold symmetrical cyclo-β-peptide. (a) Antiparallel hydrogen bonding formation where N2 and N3 atoms of the triazole ring serve as a hydrogen bond acceptor; (b) cartoon representation for parallel stacking. Only peptide backbone is shown in (a), and one sugar moiety and isopropylidene ring residue are omitted in (b) for the sake of clarity.

stacking should have given rise to a double set of signals. But in case of one tubular sheet with higher order aggregation, a unique set of signals can be retained. This is in consonance with previous quantum mechanical energy calculations[13] on cyclic D-,L-α-peptides where the existence of two β-sheet hydrogen bondings in one tubular sheet structure has been proposed.

The present study therefore reports the preparation of a chiral pseudo dipeptide derived building block from furanoid sugar molecules and its use to generate a hybrid triazole/amide macrocycle by cyclooligomerization. We have also established, by NMR and molecular modeling studies, the novel conformation of the triazole/amide macrocycle, which resembles the D-,L-amino acid based cyclic peptides with identical backbone chirality. The formation of a well-defined tubular nanostructure was suggested to have occurred by cross-strand antiparallel backbone to backbone intermolecular hydrogen bonding involving amide NH and triazole N2/N3 as well as by parallel stacking via amide NH and amide carbonyl oxygen H-bonding. Although several literature reports suggest that two types of β-sheet hydrogen bondings can coexist, this is the first experimental demonstration of this unique mode of solution phase self-assembly. NMR, ESI-MS, FT-IR, TEM, and SEM studies revealed the formation of hierarchical

(13) Qu, W; Tan, H; Chen, G; Liu, R. *Int. J. Quantum Chem.* **2010**, *110*, 1648.

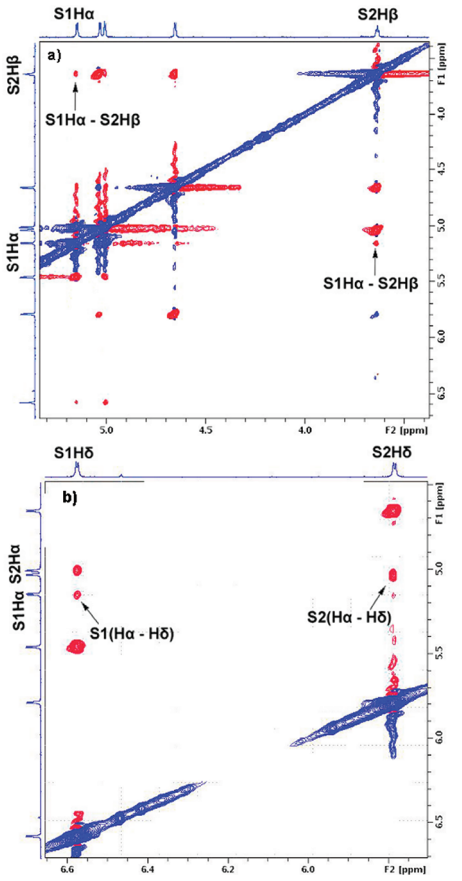


Figure 4. Selected region of ROESY spectrum of the self-assembled macrocycle: (a) antiparallel heterostacking mode revealed by cross-peaks between S1CαH and S2CβH signals and (b) parallel homostacking mode by S1CδH and S2CδH cross-peaks (600 MHz, 298K, 2:3 $CDCl_3/CCl_4$).

organization. The X-ray crystallographic study of appropriate derivatives and anion binding properties of this type of macrocycle as well as of its larger derivatives derived by functionalizing the sugar moiety will be taken up in future.

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Supporting Information Available. Detailed experimental procedure, spectral data, and TEM, SEM images. This material is available free of charge via the Internet at http://pubs.acs.org.