Organic LETTERS

Letter

pubs.acs.org/OrgLett

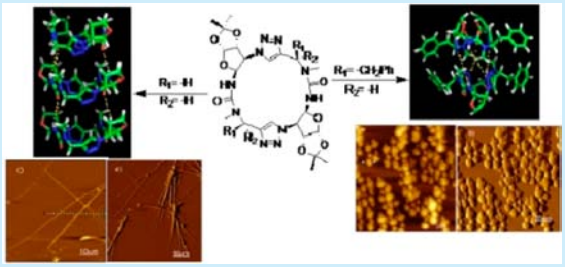
Rational Construction of Triazole/Urea Based Peptidomimetic Macrocycles as Pseudocyclo-β-peptides and Studies on Their Chirality Controlled Self-Assembly

Abhijit Ghorai, Samanth Reddy K, Basudeb Achari, and Partha Chattopadhyay*

Chemistry Division, CSIR-Indian Institute of Chemical Biology, Kolkata, 700032, India

Supporting Information

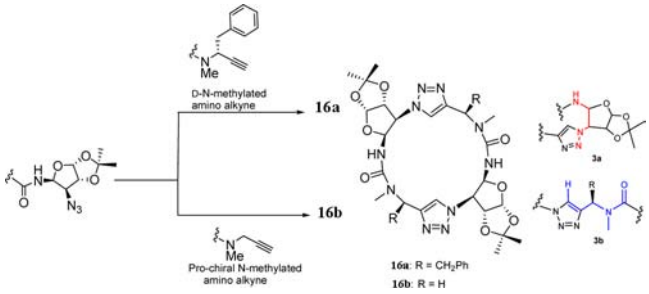
ABSTRACT: A tandem macro-dimerization reaction via a Cu(I) catalyzed azide/alkyne cycloaddition reaction has been employed to construct triazole/urea based peptidomimetic macrocycles considered as pseudocyclo-β-peptides. Introduction of one particular chirality in the peptide backbone can alter the conformation as well as nature of self-assembly from cyclic D-,L-,α-peptide to cyclo-β-peptide. One of them (**16a**) forms antiparallel dimers while the other (**16b**) undergoes higher order aggregation to form a nanorod structure.



Peptides are versatile units for the construction of H-bonded tubular assemblies and other biomimetic materials with potentially useful applications.[1] The design of unnatural amino acid based peptidomimetic macrocycles with predictable self-assembly patterns (i.e., organic nanotube forming) and function has, in particular, attracted considerable attention over the past two decades owing to the diversity in size and shape, easy access, and biocompatibility of such macrocycles. Among these, urea/amide based macrocyclic hybrids proved to be anion selective transporters in biological membranes, with numerous anticipated applications in catalysis, biotechnology, and material science.[2−4] The attractive features of this class of molecules prompted us to design specific kinds of macrocycles (parallel and antiparallel units in terms of functional groups) for future exploration of the importance of macrodipoles in anion binding, anion transport, and voltage gating.[2]

Several variations have been made in cyclic D-,L-,α-peptides as well as β-peptides to produce selective ion channels.[3b,4a,5] Cyclic β-peptides are conformationally more stable and possess an additional CH_2 which imparts flexibility to the ring toward self-assembly. Recently we succeeded in designing various regioisomers of cyclic-β-peptides incorporating 1,4-linked 1,2,3-triazole moieties to generate different (parallel and antiparallel) peptidomimetic macrocycles from carbohydrate precursors which undergo self-assembly to form organic nanotubes, but in solvents of varying polarity.[6] We envisioned that, by retaining the (1,4)-linked triazole in the backbone, urea should be a suitable replacement for the other amide groups to create greater divergence in macro-dipoles of these classes of compounds. The dipole moments of ureas exceed those of amides (4.8−4.9 D for simple symmetrical aliphatic ureas versus 3.7−3.9 D for simple amides),[2] while urea shares a number of features with the amide linkage, namely, rigidity, planarity, polarity, and hydrogen bonding capacity. As urea/amide based peptidomimetic macrocycles have been reported to display a good anion binding property, we expected to find new anion binding peptidomimetic macrocycles during this investigation (Scheme 1).[2,4]

Scheme 1. Compounds 16a and 16b are Peptidomimetic Macrocycles of which 3a and 3b are the Pseudo-β-amino Acid Residues



Our long-standing interest in exploiting carbohydrate derivatives as chiral synthons propelled us to synthesize macrocyclic triazole/urea oligomers using carbohydrate precursors. Considering one NH of urea as equivalent to a methylene group, we chose the achiral *N*-methyl propargyl amine and two of its enantiomeric derivatives[7] (based on an α-amino acid) as the nonsugar component to construct macrocyclic triazole/urea oligomers.

Although the strategy of modifying a partial peptide backbone by *N*-methylation has already been used to study the thermodynamics of nanotube formation via truncated stacks (i.e., H-bonded dimers), our initial approach was to promote cyclo-oligomerization of linear *N*-methylated ureido azido

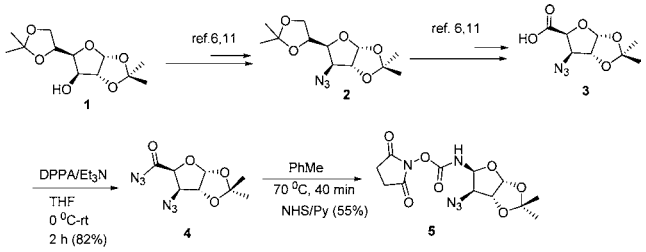
Received: April 23, 2014
Published: June 4, 2014

dx.doi.org/10.1021/ol501172d | *Org. Lett.* 2014, 16, 3196−3199

alkyne precursors to access C_2 symmetric triazole/urea based peptidomimetic macrocycles. The stability of rotameric conformations of the *cis*-β-furanoid sugar moiety prompted the use of *N*-methyl propargyl amine as the achiral unit and *N*-methylated D-benzyl propargyl amine as its chiral analogue. Methylation was done only on the amino alkyne specifically to investigate the conformational correlation of the remaining ureido-NH with the triazole N2/N3 atoms (equivalent to the carbonyl group of an amide bond) and also to analyze the nature of self-assembly of the peptidomimetic macrocycle resulting from that conformation. Our study highlights the design and successful synthesis of two novel *N*-methylated triazole/ureido based peptidomimetic macrocycles via Cu(I) catalyzed tandem dimerization of linear *N*-methylated-(ureido)-azido-alkyne precursors and the observation of their different self-assembly properties in solution phase. To our delight, the study revealed that the chirality element of a peptidomimetic macrocycle can bridge two homologous conformations of cyclic peptides and also encourage self-assembly reminiscent either of cyclo-β-peptides or of cyclic *N*-methylated D-,L-α-peptides/cyclic α,γ-peptides which form truncated dimers in solution phase as well as in the solid phase.

The linear ureido-azido alkynes were prepared by coupling the common intermediate *cis*-β-furanoid sugar azido succinimidyl ester **5** with two different *N*-methylated amino alkynes such as the chiral D-phenyl alanine derived *N*-methylated amino alkyne **10** and *N*-methylated propargyl amine **13** (Schemes 2,3). The Boc-D-phenyl alanine **6** based propargyl amine

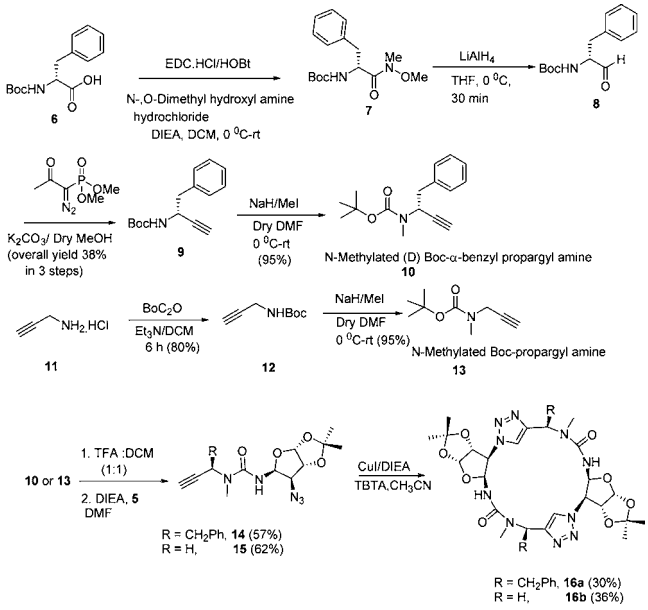
Scheme 2. Synthesis of Activated *cis*-Furanoid-β-azido Succinimidyl Carbamate



derivative was synthesized by the Ohira–Bestmann reaction[8a] of the corresponding aldehyde **8**, which was readily accessible by reduction of the Weinreb[8b] amide **7** of Boc-D-phenyl alanine with $LiAlH_4$. The *N*-methylated protected alkyne **10** was prepared by the reaction of methyl iodide and sodium hydride with the Boc protected amino alkyne **9**. Intermediate **5** was synthesized from acetonide protected D-glucose **1** to give (Scheme 2), via the azide-diacetonide **2**, the *cis*-β-furanoid sugar azido acid **3**, which was treated with DPPA/Et_3N to get **4** and then subjected to Curtius rearrangement to get the isocyanate. Activation of the latter by *N*-hydroxy succinimide afforded the desired azido succinimidyl carbamate derivative **5** smoothly.

Two different linear ureido-azido-alkyne precursors **14** and **15** were obtained by coupling the two different *N*-methylated amino alkynes separately with intermediate **5** under basic conditions. These two azido-alkyne derivatives were then treated with CuI/DIEA in the presence of a catalytic amount of TBTA to provide the desired peptidomimetic macrocycles **16a** and **16b**. Only dimeric products (ESI-MS) were isolated by HPLC though evidence for the formation of higher oligomers in traces was obtained.

Scheme 3. Synthesis of Peptidomimetic Macrocycles 16a and 16b



Conformational analysis of the two peptidomimetic macrocycles was carried out by molecular modeling combined with findings from proton as well as multidimensional NMR spectroscopy such as DQF-COSY and ROESY. ^{1}H NMR spectra of **16a** in polar and nonpolar solvents (DMSO-d_6, CCl_4–$CDCl_3$, or MeOH-d_4) are well-defined, reflecting a high degree of C_2 symmetry. The observed coupling constant $J_{NH,C\alpha H(S)}$ is approximately 4.6 Hz, implying a pseudo positive φ angle (i.e., 60°) which is generally accessible with D-α-amino acid residues.[2,6] Strong ROESY cross peaks between N-Me and N-H indicate the symmetrical (*trans*, *trans*) nature of the molecule like those of aliphatic ureas.[2] Molecular modeling and ROESY spectroscopic studies revealed that the peptidomimetic macrocycle tends to minimize nonbonded intramolecular side chain–side chain and side chain–backbone interactions by adopting a flat ring-shaped conformation (Figure 1). The urea and triazole backbones are perpendicular to the mean plane of the peptide ring, where N2/N3 and urea carbonyl (or urea-NH

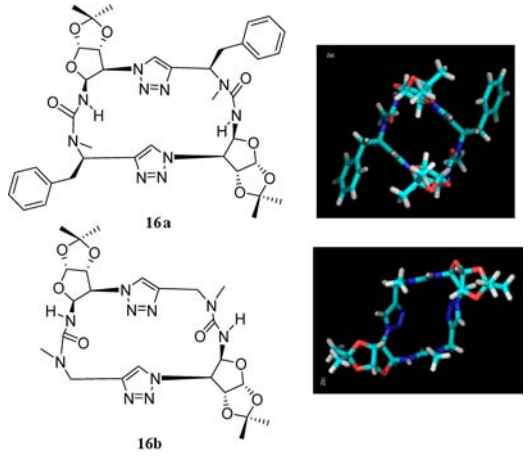


Figure 1. Energy minimized structures of peptidomimetic macrocycles **16a** and **16b**: (a) Functional group mimics of cyclic D-,L-α-peptides. (b) Mimics of cyclo-β-peptides.[11]

dx.doi.org/10.1021/ol501172d | *Org. Lett.* 2014, 16, 3196–3199

and triazole CH) groups[9] are alternatively oriented along opposite faces of the pseudo peptide backbone (Figure 2a).

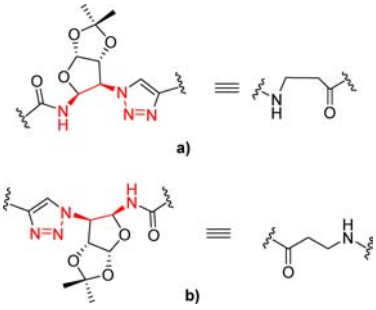


Figure 2. Triazole and amide equivalency: (a) −NH and (N2−N3) in same direction like L-α-amino acid residue in cyclic D-,L-α-peptides; (b) −NH and (N2−N3) in opposite directions like β-amino acids in the cyclo-β-peptide conformation.[11]

In analogy with partially *N*-methylated D-,L-α-peptides and α,γ-peptides, the cyclic urea/triazole oligomer **16a** was thought to self-assemble in an antiparallel manner to form H-bonded dimers maintained by a set of four strong H-bonds involving urea NH groups and N2−N3 atoms (equivalent to amide carbonyl groups)[8] of the triazole ring of identical pseudo-β-amino acid residues (homostacking) (Figure 3). Another

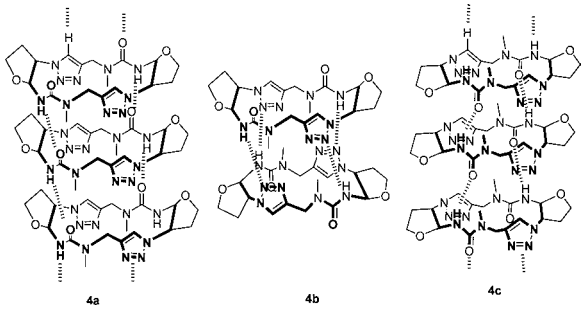


Figure 3. Possibility of self-organization of **16a** in solution phase: (**4a**) parallel stacking via (β-D,β-L) mode of H-bonding to form higher order aggregation; (**4b**) antiparallel mode of hydrogen bonding leading to truncated-dimer formation; (**4c**) parallel stacking of **16b** via (β,β)-H bonding similar to cyclo-β-peptides.[10]

possibility of self-organization to form organic nanotubes via heterostacking can involve parallel (β-D,β-L) H-bonding of NH to carbonyl C═O and of CH to (N2−N3) of (1,4)-linked triazole due to the identity of chirality throughout the peptide backbone as noted by us earlier.[6]

The β-sheet-like arrangement was confirmed by the FTIR spectrum which shows amide A, I, and II bands at 3304, 1645, and 1520 cm^{-1} in $CHCl_3$ (15 mM) as observed with previously reported cyclic peptides as well peptidomimetic macrocycles.[2,6]

To gain insight into oligomer formation, we performed ^1H as well as multidimensional NMR experiments. A substantial downfield shift (>0.1 ppm) of the CHα proton of the sugar moiety reflects a dimeric β-structure formation by **16a** in a nonpolar solvent such as $CDCl_3$ or (2:3) $CDCl_3$:CCl_4 (Figure 4). Concentration-dependent NMR experiments provided further evidence for the formation of H-bonded dimers in solution in $CDCl_3$. In agreement with the proposed antiparallel dimerization scheme, the proton resonance of ureido NH but not of triazole CH experienced a significant downfield shift

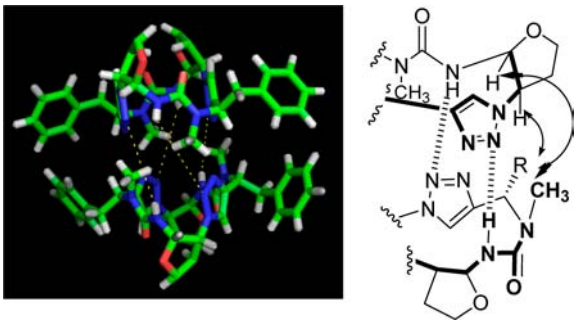


Figure 4. A typical side view of H-bonded dimer **16a** by molecular modeling. The right part is shown for Roesy interpretation in (2:3) $CDCl_3$:CCl_4 (600 MHz, 298 K).

(from 4.43 to 4.20 ppm at 223 K) as the concentration increased from 1 to 50 mM.

The retention of the unique pattern of proton NMR is likely due to fast equilibrium on the NMR time scale.[2,6,10] Definitive evidence in favor of antiparallel dimerization came from the observed ROESY cross-peaks between the N-Me and α-CH proton as well as β-CH proton signals of the sugar moiety in (2:3) $CDCl_3$:CCl_4 (Figure 5).

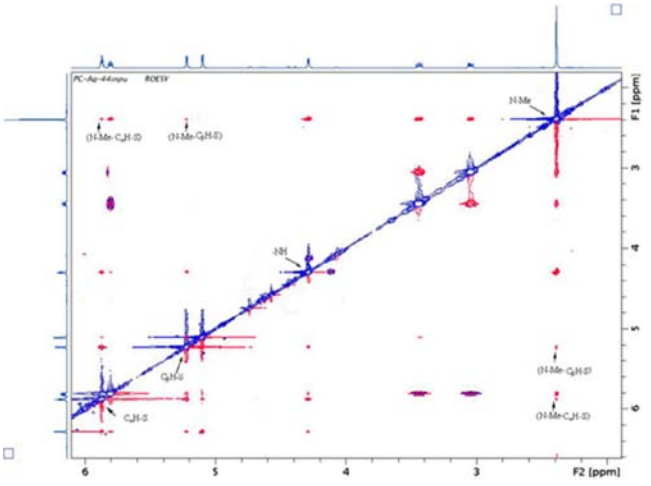


Figure 5. Selected region of the ROESY spectrum of self-assembled macrocycle **16a**, showing dimer formation through cross-peaks of N-Me with *S*-CH_α as well as *S*-CH_β (600 MHz, 298 K, 2:3 $CDCl_3$:CCl_4).

To support this conclusion, the study of AFM morphology on a mica foil was employed. For this, compound **16a** was dissolved to a concentration of 50 μM in a nonpolar solvent such as (2:3) $CDCl_3$:CCl_4. The nonpolar solvent was chosen because the dipole moment of the ensemble is not expected to be large, as the triazole moiety and the urea linkage are oriented in opposite directions. Interestingly this showed several ball-like composites (reflecting several numbers of dimers in solution phase) stacking together instead of any nanotube formation (via β-L and pseudo β-D H-bonding). The cyclic urea/triazole oligomer **16a** was therefore concluded to self-assemble in an antiparallel manner to form H-bonded dimers as discussed above, by analogy with partially *N*-methylated cyclic D-,L-α-peptides and cyclic-(α,γ)-peptides.

Substituting the chiral *N*-methylated α-propargyl amine in urea/triazole cyclodimers with an achiral unit as in **16b** had

dx.doi.org/10.1021/ol501172d | *Org. Lett.* 2014, 16, 3196−3199

dramatic consequences on both ring geometry and self-assembly properties of the resulting cyclodimer.

^{1}H NMR spectra of this macrocycle in nonpolar ($CDCl_3$) as well as polar (CD_3CN) solvents are also well-defined and predictive of a C_2 symmetric nature. But in contrast to **16a**, the ureido NH proton chemical shift of 6.12 in CD_3CN with a large coupling constant of 10.2 Hz testified to an antiperiplanar arrangement of the ureido-amide proton with the neighboring $^{\alpha}$CH of the sugar moiety. Therefore, the urea-triazole backbone should be perpendicular to the mean plane of the peptide ring where N2/N3 (equivalent to a carbonyl group) and a urea carbonyl (or urea-NH and triazole CH) are oriented along the same face of the pseudo peptide backbone which is similar to any self-assembling cyclic-β-peptide conformation (Figures 1 and 2b). The ^{1}H NMR recorded in a nonpolar solvent such as $CDCl_3$ displayed significant broadening of all proton signals of the pseudocyclo-β-peptide which is attributed to intermolecular hydrogen bond mediated aggregation giving rise to multiple supramolecular species exchanging on the NMR time scale. The Roesy cross peaks between S-CH_{β} and S-CH_{δ} (see Supporting Information) provide definitive evidence of parallel stacking to form organic nanotubes in CD_3CN (containing 2% H_2O) as is observed with typical cyclo-β-peptides as well as peptidomimetic macrocycles.[6,10] The detection of another level of hierarchical organization, the formation of well-defined tubular nanostructures (20 nm to 1.2 mm diameter) observed by TEM as well as AFM imaging (see Supporting Information) in polar solvents such as CH_3CN, reflects the large dipole moment of the macrocyclic self-ensemble.

In summary, we have designed and synthesized two novel classes of (1,4)-linked triazole/urea based pseudo cyclic peptides (peptidomimetic macrocycles) by applying Cu(I) catalyzed tandem dimerization−click chemistry on linear *N*-methylated ureido-(azido/alkyne) precursors. One of them (**16a**), which is built up entirely of chiral units, appears conformationally homologous to *N*-methylated cyclic D-,L-α-peptides in terms of functional group while retaining the backbone chirality.

In solution phase self-assembly behavior, it exactly resembles cyclic D-,L-α-peptides as well as cyclic α,γ-peptides. The other compound (**16b**), constructed from achiral alkyne units, displays a typical cyclo-β-peptide conformation and hierarchical organization conducive for nanotube formation which has been confirmed by NMR, FT-IR, TEM, and AFM study. The anion binding properties of these novel macrocycles will be the subject of future studies.

■ ASSOCIATED CONTENT

Supporting Information

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

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: partha@iicb.res.in.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank CSIR-India for research fellowship (to A.G.) and Dept of Science and Technology, Govt. of West Bengal, India for financial support.

■ REFERENCES

(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) (a) Hennig, A.; Fischer, L.; Guichard, G.; Matile, S. *J. Am. Chem. Soc.* **2009**, *131*, 16889. (b) Fischer, L.; Decossas, M.; Briand, J.-P.; Didierjean, C.; Guichard, G. *Angew. Chem., Int. Ed.* **2009**, *48*, 1625 and references therein. (c) Fischer, L.; Guichard, G. *Org. Biomol. Chem.* **2010**, *8*, 3101.

(3) (a) Patzke, G. R.; Krumeich, F.; Nesper, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 2446. (b) Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R. *Angew. Chem., Int. Ed.* **2001**, *40*, 988. (c) Ajayan, P. M.; O. Zhou, Z. *Top. Appl. Phys.* **2001**, *80*, 391.

(4) (a) Clark, T. D.; Buehler, L. K.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 651. (b) Clark, T. D.; Kobayashi, J. M.; Isler, M. P.; McRee, D. E.; Ghadiri, M. R. *J. Am. Chem. Soc.* **1998**, *120*, 8949. (c) Amorin, M.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2003**, *125*, 2844. (d) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 9372. (e) Maarseveen, J.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503−4506. (f) García-Fandiño, R.; Granja, J. R.; D'Abramo, M.; Orozco, M. *J. Am. Chem. Soc.* **2009**, *131*, 15678.

(5) (a) Wang, D.; Guo, L.; Zhang, J.; Jones, L. R.; Chen, Z.; Pritchard, C.; Roeske, R. W. C. *J. Pept. Res.* **2001**, *57*, 301. (b) Seebach, D.; Matthews, J. L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCuser, L. B. *Helv. Chim. Acta* **1997**, *80*, 173.

(6) (a) Ghorai, A.; Padmanaban, E.; Mukhopadhyay, C.; Achari, B.; Chattopadhyay, P. *Chem. Commun.* **2012**, *48*, 11975. (b) Ghorai, A.; Gayen, A.; Kulsi, G.; Padmanaban, E.; Laskar, A.; Achari, B.; Mukhopadhyay, C.; Chattopadhyay, P. *Org. Lett.* **2011**, *13*, 5512−5515.

(7) Use of *N*-methyl L-phenyl alanine derived propargyl amine failed to provide any cyclic oligomer presumably due to the disfavored conformer of the peptide backbone unit (see Supporting Information).

(8) (a) Ohira, S. *Synth. Commun.* **1989**, *19*, 561. (b) De Luca, L.; Giacomelli, G.; Taddei, M. *J. Org. Chem.* **2001**, *66*, 2534.

(9) (a) Angell, Yu L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674. (b) Brik, J.; Alexandratos, A.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *ChemBioChem.* **2005**, *6*, 1167.

(10) See Supporting Information.

(11) (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.