JOC*Note*

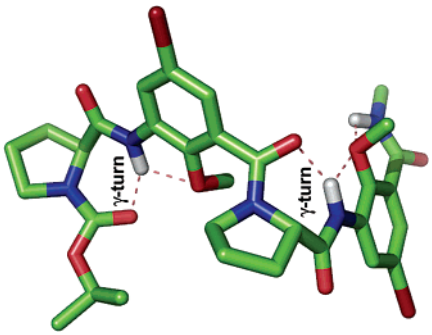
Enforcing Periodic Secondary Structures in Hybrid Peptides: A Novel Hybrid Foldamer Containing Periodic γ-Turn Motifs

Pranjal K. Baruah,[†] N. K. Sreedevi,[†] Rajesh Gonnade,[‡] Sapna Ravindranathan,[§] Krishnan Damodaran,[⊥] Hans-Jörg Hofmann,*[,||] and Gangadhar J. Sanjayan*[,†]

Division of Organic Synthesis, Central Material Characterization Division, and Central NMR Facility, National Chemical Laboratory, Dr. Homi Bhabha Road, Pune 411 008, India, Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, and Universität Leipzig, Institut für Biochemie, Brüderstrasse 34, D-04103 Leipzig, Germany

hofmann@uni-leipzig.de; gj.sanjayan@ncl.res.in

Received October 2, 2006



This note describes the design, synthesis, and conformational studies of a novel hybrid foldamer that adopts a definite compact, three-dimensional structure determined by a combined effect of the special conformational properties of the foldamer constituents. The striking feature of this de novo designed foldamer is its ability to display periodic γ-turn conformations stabilized by intramolecular hydrogen bonds. Conformational investigations by single-crystal X-ray studies, solution-state NMR, and ab initio MO theory at the HF/6-31G* level strongly support the prevalence of γ-turn motifs in both the di- and tetrapeptide foldamers, which are presumably stabilized by bifurcated hydrogen bonds in the solid and solution states. The strategy disclosed herein for the construction of hybrid foldamers with periodic γ-turn motifs has the potential to significantly augment the conformational space available for foldamer design with diverse backbone structures and conformations.

Over the past three decades, chemists have made key strides in learning the fundamental rules of folding propensities of peptides and proteins involving noncovalent interactions.[1] The mystery of how a protein sequence specifies a unique structure and function has intrigued chemists leading to the design and development of foldamers,[2] which are synthetic oligomers with a definite conformational backbone structure. The foldamer approach has been extensively utilized generating diverse sets of structures which are able to mimic secondary structure elements like β-turns, helices, and β-pleated sheets.[3] The driving force for these efforts has been the possibility of achieving suitable templates for the design of biologically active molecules that compete for a variety of protein−protein[4] and protein−membrane interactions, respectively.[5] These synthetic oligomers may provide excellent starting points for the elaboration of peptide mimics that could be designed only with difficulties on the basis of small-molecule scaffolds.[6] To extend the repertoire of foldamer design, foldamers containing different residues of independent conformational preferences were recently suggested. For instance, several groups demonstrated that α,β-hybrid peptides composed of alternately changing α- and β-amino acid constituents showed convincing evidence for the formation of special helix types.[7] We ourselves have recently provided theoretical insights into the helix formation propensities in α,β-, α,γ-, and β,γ-hybrid peptides.[8] Furthermore, the potential of using unconventional foldamer building blocks for the design of protein secondary structure mimetics has also been described.[9] In this note, we describe a foldamer having proline (Pro) and 3-amino-5-bromo-2-methoxy benzoic acid (Amb) as alternating subunits that forms repeating γ-turn conformations. Although not so ubiquitous as β-turns, γ-turns consisting of three amino acid residues have been implicated in several important biologi-

[†] Division of Organic Synthesis, National Chemical Laboratory.
[‡] Central Material Characterization Division, National Chemical Laboratory.
[§] Central NMR Facility, National Chemical Laboratory.
[⊥] Department of Chemistry, University of Pittsburgh.
[||] Universität Leipzig, Institut für Biochemie.

(1) (a) Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev.* **2001**, *101*, 3131−3152. (b) Shi, Z.; Chen, K.; Liu, Z.; Kallenbach, N. R. *Chem. Rev.* **2006**, *106*, 1877−1897. (c) MacKenzie, K. R. *Chem. Rev.* **2006**, *106*, 1931−1977.

(2) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173−180.

(3) For representative reviews, see: (a) Smith, M. D.; Fleet, G. W. J. *J. Peptide Sci.* **1999**, *5*, 425−441. (b) Kirschenbaum, K.; Zuckerman, R. N.; Dill, D. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 530−535. (c) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232. (d) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893−4011. (e) Cubberley, M. S.; Iverson, B. L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 650−653. (f) Stigers, K. D.; Soth, M. J.; Nowick, J. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 714−723. (g) Sanford, A. R.; Yamato, K.; Yang, X.; Yuan, L.; Han, Y.; Gong, B. *Eur. J. Biochem.* **2004**, *271*, 1416−1425. (h) Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, *1*, 1111−1239.

(4) Kritzer, J. A.; Hodsdon, M. E.; Schepartz, A. *J. Am. Chem. Soc.* **2005**, *127*, 4118−4119.

(5) Liu, D.; Choi, S.; Chen, B.; Doerksen, R. J.; Clements, D. J.; Winkler, J. D.; Klein, M. L.; DeGrado, W. F. *Angew. Chem., Int. Ed.* **2004**, *43*, 1158−1162.

(6) (a) Choi, S.; Clements, D. J.; Pophristic, V.; Ivanov, V.; Vemparala, S.; Bennett, J. S.; Klein, M. L.; Winkler, J. D.; DeGrado, D. F. *Angew. Chem., Int. Ed.* **2005**, *44*, 6685−6689. (b) Baldauf, C.; Guenther, R.; Hofmann, H.-J. *J. Org. Chem.* **2005**, *70*, 5351−5361.

(7) (a) Hayen, A.; Schmitt, M. A.; Ngassa, F. N.; Thomasson, K. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 505−510. (b) De Pol, S.; Zorn, C.; Klein, C. D.; Zerbe, O.; Reiser, O. *Angew. Chem., Int. Ed.* **2004**, *43*, 511−514. (c) Sharma, G. V. M.; Nagendar, P.; Jayaprakash, P.; Krishna, P. R.; Ramakrishna, K. V. S.; Kunwar, A. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 5878−5882.

(8) (a) Baldauf, C.; Gunther, R.; Hofmann, H.-J. *Biopolymers* **2006**, *84*, 408−413. (b) Baldauf, C.; Gunther, R.; Hofmann, H.-J. *J. Org. Chem.* **2006**, *71*, 1200−1208.

(9) Acrylamide oligomers have been shown to adopt β-sheet-like structures, see: Kendhale, A.; Gonnade, R.; Rajamohanan, P. R.; Sanjayan. G. J. *Chem. Commun.* **2006**, 2756−2758.

10.1021/jo062032w CCC: $37.00
Published on Web 12/23/2006

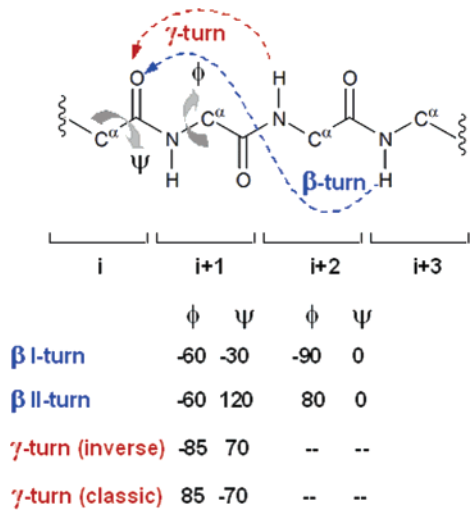
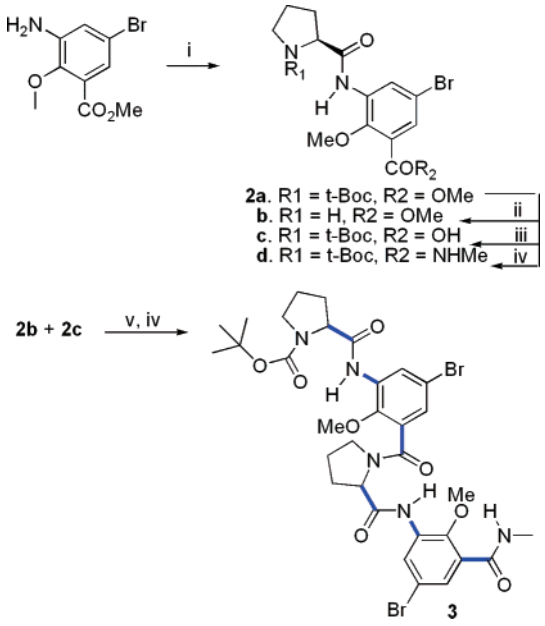


FIGURE 1. Schematic representation of the intramolecular hydrogen bonding interaction that stabilizes reverse turns such as the 10-membered hydrogen-bonded ring in β-turns ($i \leftarrow i + 3$) and the 7-membered hydrogen-bonded ring in γ-turns ($i \leftarrow i + 2$). The ideal values for the torsion angles ϕ and ψ (highlighted in bold gray arrows) for the inverse and classic γ-turns and the most common types of β-turns, βI and βII, are indicated.

cal events.[10] They have been frequently postulated to represent an important feature of peptide secondary structure as determined by ^{1}H NMR,[11] X-ray,[12] and molecular modeling.[13] Two types of γ-turns have been characterized, the classical and the inverse ones, which are defined by the torsion angles of the central amino acid residue (Figure 1).

Whereas inverse γ-turns are more frequently observed in proteins,[14] classical γ-turns are rather rare.[15] It has been challenging to investigate the role of γ-turns in protein−peptide recognition because they seldom exist as stable conformers in short, linear peptides, but have to be constrained by ring formation (backbone cyclization)[16,17] or by the closer positioning of proline residues to highly constrained cyclopropane amino acid residues.[18]

SCHEME 1. Synthesis of Hybrid Foldamers[a]



[a] Reagents and conditions: (i) Boc-Pro-OH, DIPEA, TBTU, MeCN, rt, 12 h; (ii) dry HCl (gas), dioxane, rt, 15 min; (iii) LiOH, MeOH, rt, 24h; (iv) methanolic $MeNH_2$, 24 h, rt; (v) DIPEA, TBTU, MeCN, rt, 12 h. Note: To facilitate identification, the conformational restriction imposed by the Pro and Amb residues in **3** is highlighted in blue bold bonds.

Design Principles. We designed the Pro-Amb motif-based foldamer anticipating that the corresponding oligomers would adopt a definite three-dimensional structure determined by a combined conformational restriction imposed by both the Pro and the Amb residues (highlighted in bold blue bonds in Scheme 1). Whereas the Pro residue, with its torsion angle ϕ constrained at about −60°, is known to promote PPII helical conformation in its homo oligomers,[19] analogues of the backbone-rigidified aromatic amino acid residue Amb are known to induce a crescent conformation in the oligomers[3g,20] via localized S(5) and S(6) type[21] hydrogen bonding interactions. Thus we reasoned that heterooligomers made of the Pro-Amb motif would also display a conformationally rigid three-dimensional structure. Structural studies (vide infra) indeed showed that the Pro-Amb motif-based tetrapeptide foldamer **3** folds into a well-defined, compact, three-dimensional structure with periodic γ-turn motifs stabilized by two sets of intramolecular bifurcated hydrogen bonds.

Results and Discussion. The Pro-Amb motif-based foldamer **3** was assembled from the Boc-Pro-Amb-OMe building block **2a** by using "segment doubling strategy"[22] (Scheme 1). **2a**, in turn, was synthesized by coupling the protected amino acids Pro and Amb, using TBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate) as a coupling agent and DIPEA (*N*,*N*-diisopropylethylamine) as the base (experimental details in the Supporting information).

(10) (a) Rosenstroem, U.; Skoeld, C.; Plouffe, B.; Beaudry, H.; Lindeberg, G.; Botros, M.; Nyberg, F.; Wolf, G.; Karlen, A.; Gallo-Payet, N.; Hallberg, A. *J. Med. Chem.* **2005**, *48*, 4009−4024. (b) Hedenstroem, M.; Yuan, Z. Q.; Brickmann, K.; Carlsson, J.; Ekholm, K.; Johansson, B.; Kreutz, E.; Nilsson, A.; Sethson, I.; Kihlberg, J. *J. Med. Chem.* **2002**, *45*, 2501−2511. (c) Yuan, Z. Q.; Blomberg, D.; Sethson, I.; Brickmann, K.; Ekholm, K.; Johansson, B.; Nilsson, A.; Kihlberg, J. *J. Med. Chem.* **2002**, *45*, 2512−2519.

(11) Narutis, V. P.; Kopple, K. D. *Biochemistry* **1983**, *22*, 6233−6239.

(12) Loosi, H. R.; Kessler, H.; Oschkinat, H.; Weber, H. P.; Petcher, T. J.; Widmer, A. *Helv. Chim. Acta.* **1985**, *68*, 682−704.

(13) Hagler, A. T.; Osguthorpe, D. J.; Dauber-Osguthorpe, P.; Hempel, J. C. *Science* **1985**, *227*, 1309−1315.

(14) Frishman, D.; Argos, P. *Proteins* **1995**, *23*, 566−579.

(15) Milner-White, E. J. *J. Mol. Biol.* **1990**, *216*, 386−397.

(16) For heterocyclic γ-turns, see: (a) Etzkorn, F. A.; Travins, J. M.; Hart, S. A. *Adv. Amino Acid Mimetics Peptidomimetics* **1999**, *2*, 125−163. (b) Alkorta, I.; Suarez, M. L.; Herranz, R.; Gonzalez-Muniz, R.; Garcia-Lopez, M. T. *J. Mol. Model.* **1996**, *2*, 16−25. (c) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. S.; Hwang, S. M.; Kopple, K. D.; Koster, P. F.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Vasko, J. A.; Wong, A.; Huffman, W. F. *J. Med. Chem.* **1992**, *35*, 3970−3972. (d) Newlander, K. A.; Callahan, J. F.; Moore, M. L.; Tomaszek, T. A., Jr.; Huffman, W. F. *J. Med. Chem.* **1993**, *36*, 2321−2331. (e) Callahan, J. F.; Newlander, K. A.; Burgess, J. L.; Eggleston, D. S.; Nichols, A.; Wong, A.; Huffman, W. F. *Tetrahedron* **1993**, *49*, 3479−3488.

(17) For γ-turns induced by 2,3-methanoamino acids, see: (a) Burgess, K.; Ho, K.-K.; Pettitt, B. *J. Am. Chem. Soc.* **1994**, *116*, 799−800. (b) Burgess, K.; Ke, C.-Y. *J. Org. Chem.* **1996**, *61*, 8627−8631. (c) Moye-Sherman, D.; Jin, S.; Li, S.; Welch, M. B.; Reibenspies, J.; Burgess, K. *Chem.-Eur. J.* **1999**, *5*, 2730−2739.

(18) Jimenez, A. I.; Ballano, G.; Cativiela, C. *Angew. Chem., Int. Ed.* **2005**, *44*, 396−399.

(19) Creamer, T. P.; Campbell, M. N. *Adv. Protein Chem.* **2002**, *62*, 263−282.

(20) (a) Zhu, J.; Parra, R. D.; Zeng, H.; Jankun, E. S.; Zeng, X. C.; Gong, B. *J. Am. Chem. Soc.* **2000**, *122*, 4219−4220. (b) Gong, B. *Chem.-Eur. J.* **2001**, *7*, 4336−4342.

(21) Etter, M. C. *Acc. Chem. Res.* **1990**, *23*, 120−126.

(22) Wender, P. A.; Jessop, T. C.; Pattabiraman, K.; Pelkey, E. T.; VanDeusen, C. L. *Org. Lett.* **2001**, *3*, 3229−3232.

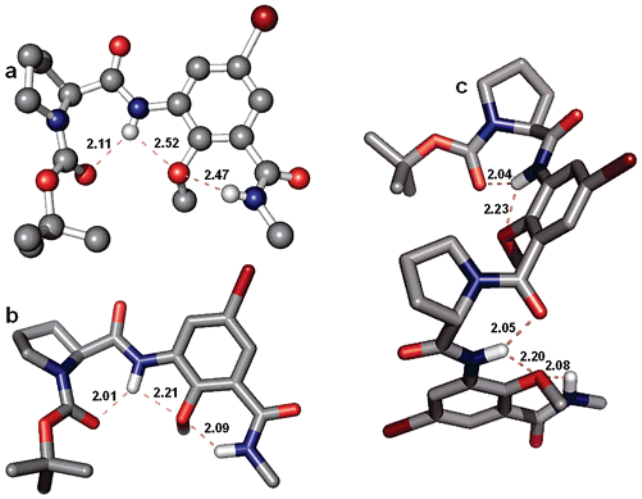


FIGURE 2. Conformation of the foldamers **2d** and **3** showing bifurcated H-bond stabilized γ-turn motifs: (a) single-crystal X-ray structure of **2d**; (b) conformation of **2d** at the HF/6-31G* level of ab initio MO theory; and (c) conformation of **3** at the HF/6-31G* level of ab initio MO theory.

Structural studies employing NMR and quantum chemical methods show that the Pro-Amb motif-based tetrapeptide foldamer **3** folds into a well-defined compact three-dimensional structure with periodic γ-turn motifs. The detailed analysis indicates that the prolylamide NH group is involved not only in the S(7)-type 7-membered γ-turn formation, but also in an S(5)-type hydrogen bonding[21] with the Amb methoxy group. It should be noted that simple *N*-acyl prolylamide model peptides are known to exist in multiple conformational equilibrium, where the γ-turn is only one of the emerging conformers.[23] Presumably, the *O*-methoxy group of Amb in both **2d** and **3** causes further conformational restriction forming a bifurcated hydrogen-bonded network that favors the population of the γ-turn conformation. This bifurcated hydrogen-bonded structural feature was already realized at the dipeptide level itself as the crystal structure of the dipeptide foldamer **2d** reveals (Figure 2a).

The hydrogen-bonding geometry of the γ-turn is well in accordance with an inverse γ-turn pattern ($\phi = -87°$ and $\psi = 53°$). The experimental structure of the dipeptide **2d** is in good agreement with the most stable conformer obtained by quantum chemical calculations employing ab initio MO theory (Figure 2b). Unfortunately, the Pro-Amb motif-based tetrapeptide foldamer **3** could not be crystallized, despite best efforts. However, it was possible to confirm the essential structural aspects by NMR data and by theoretical calculations. The theoretical conformational analysis strongly suggests the occurrence of periodic γ-turn motifs in **3**, as observed in the crystal structure of the shorter analogue **2d** (Figure 2c). This is strongly supported by 2D ROESY NMR studies in solution (500 MHz, $CDCl_3$), which indicate the prevalence of γ-turn conformations for **3** similar to that observed in the solid state of **2d** on the basis of characteristic ROE interactions. One of the most characteristic ROE interactions that can be anticipated for an 1 ← 3 (C_7) H-bonded γ-turn conformation, as observed in the solid state−state structure of **2d**, would be the requirement of dipolar coupling between proline α-C*H* vs aryl-N*H* of adjacent residues.

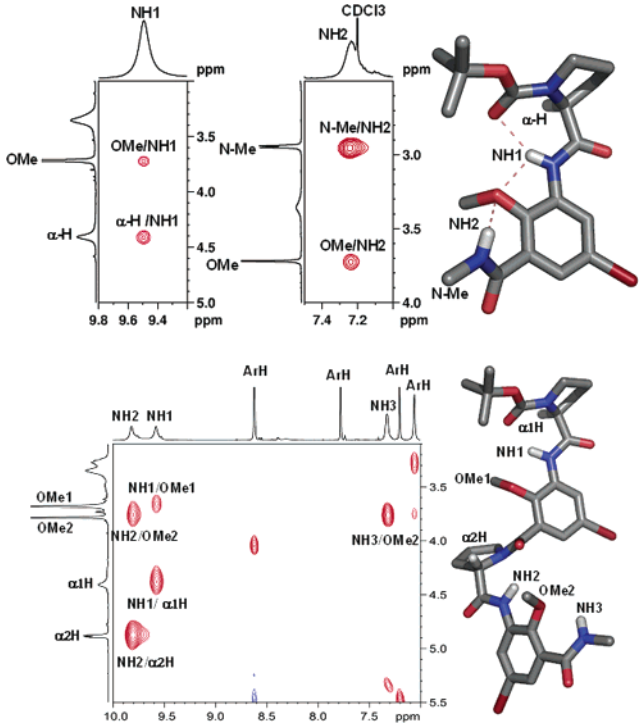


FIGURE 3. Partial 2D ROESY spectra of **2d** (top) and **3** (bottom) (500 MHz, $CDCl_3$) showing characteristic ROE interactions. The HF/6-31G* structures of **2d** and **3** with labeled atoms are also shown to facilitate signal assignments.

The analysis of the 2D ROESY data indeed revealed the existence of proline α-C*H* vs aryl-N*H* dipolar couplings of the adjacent residues in **2d** (α-CH/NH1) and in **3** (NH1/α1-CH, and NH2/ α2-CH) (Figure 3). Furthermore, the characteristic ROE interactions between aryl-N*H* and the adjacent *O*-aryloxymethyls in **2d** (OMe/NH1) and in **3** (NH1/OMe1, NH2/OMe2, and NH3/OMe2) also strongly suggest their *syn* orientation, thereby making space for the S(5) type hydrogen-bonded arrangement, a common feature in *O*-alkoxy arylamines and a prerequisite for the bifurcated hydrogen bonding.[3g,20]

To confirm that intramolecular hydrogen bonds are clearly prevalent in solution, we also performed [D_6]DMSO titration and $CDCl_3$ dilution studies of **2d** and **3** (Supporting Information). Notably, all the NH signals of both **2d** and **3** appear at the downfield region suggesting their involvement in extensive hydrogen bonding interactions. Remarkably, the protons involved in γ-turn formation show little shift when solutions of **2d** and **3** are titrated gradually with [D_6]DMSO ($\Delta\delta < 0.09$ ppm) or diluted from 100 to 1 mM ($\Delta\delta < 0.04$ ppm), suggesting their strong involvement in intramolecular hydrogen bonding.

Summarizing our results, we have designed and synthesized a novel hybrid foldamer that adopts a well-defined compact, three-dimensional architecture, which is governed by a combined conformational restriction imposed by the individual amino acids of which it is composed.[24] Conformational investigations by single-crystal X-ray studies,[25] solution state NMR, and ab initio

(23) (a) Jin, Y.; Tonan, K.; Ikawa, S. *Spectrochim. Acta, Part A* **2002**, *58*, 2795−2802. (b) Ishimoto, B.; Tonan, K.; Ikawa, S. *Spectrochim. Acta, Part A* **2000**, *56*, 201−209.

(24) Cyclic peptides containing proline−aromatic amino acid units have been reported by Kubik et al., see: (a) Otto, S.; Kubik, S. *J. Am. Chem. Soc.* **2003**, *125*, 7804−7805. (b) Heinrichs, G.; Kubik, S.; Lacour, J.; Vial, S. *J. Org. Chem.* **2005**, *70*, 4498−4501.

(25) The crystal structures were processed with the PyMOL Molecular Graphics System. Delano, W. L. *The PyMOL Molecular Graphics System*, 2004: http://www.pymol.org.

MO theory[26] strongly suggest the prevalence of γ-turn motifs in both the di- and tetrapeptide foldamers, which are presumably stabilized by strong bifurcated hydrogen bonds in the solid and solution states. The strategy disclosed herein for the construction of hybrid foldamers with periodic γ-turn motifs has the potential to significantly augment the conformational space available for foldamer design with diverse backbone structures and conformations, and will have a bearing on practical utility. Further studies are underway to extend this hybrid foldamer strategy for the construction of diverse foldamers with periodic secondary structure motifs.

Experimental Section

Crystal Data for 2d. $C_{19}H_{26}BrN_3O_5$; $M = 456.34$; crystal size, $0.28 \times 0.21 \times 0.12$ mm^3; $T = 297(2)$ K; crystal system, monoclinic; space group, $P2_1$; $a = 10.209(2)$ Å, $b = 9.5116(18)$ Å, $c = 11.486(2)$ Å, $\beta = 95.826(3)°$, $V = 1109.6(4)$ Å^3, $Z = 2$, $F(000) = 472$, d_{calc} [g cm^{-3}] = 1.366, μ [mm^{-1}] = 1.885; absorption correction, multiscan; $T_{min} = 0.6214$, $T_{max} = 0.8124$; 7929 reflections collected, 3727 unique reflections, 2933 observed reflections, 258 refined parameters; $R_1[I>2\sigma(I)] = 0.0418$, $wR_2 = 0.0961$ (all data $R = 0.0567$, $wR2 = 0.1032$); goodness of fit, 1.016; $\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3}) = 0.363, −0.264. Crystallographic data for **3** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-612864. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK.

(26) The HF/6−31G* structures (PDB format) of **2d** and **3** are available in the Supporting Information.

Acknowledgment. P.K.B. is thankful to CSIR, New Delhi, for a Senior Research Fellowship. This work was partially funded by the Department of Biotechnology (DBT), New Delhi, in-house project, NCL, and Deutsche Forschungsgemeinschaft (Project HO 2346/1-3 and SFB 610).

Supporting Information Available: Full experimental details, ^{1}H, ^{13}C, DEPT-135 NMR, dilution and titration data (tables) of **2d** and **3**, ESI mass spectra, details of quantum chemical calculations (PDF), crystal data of **2d** (CIF), and HF/6-31G* structures of **2d** and **3** (PDB). This material is available free of charge via the Internet at http://pubs.acs.org.

JO062032W