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Synthesis of Cyclic ($\alpha_2\beta$)-Tripeptides as Potential Peptide Turn Mimetics

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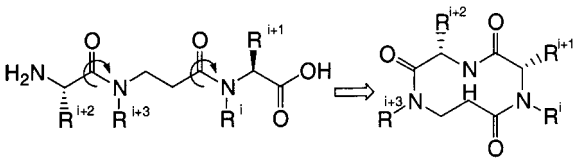
ABSTRACT



The solid-supported synthesis followed by cyclization in solution of cyclic ($\alpha_2\beta$)-tripeptides, potential peptide β-turn mimetics, is described. The cyclization takes advantage of facilitating the rotation between *trans*- and *cis*-rotamers of two amide bonds. The method is amenable to combinatorial approaches as is illustrated by the synthesis of a small array of cyclic ($\alpha_2\beta$)-tripeptides.

The β-turn is one of the important secondary structure elements in proteins. It is defined as any tetrapeptide sequence in which the $C_\alpha{}^i - C_\alpha{}^{i+3}$ distance in a nonhelical region is less than 7 Å and is often stabilized by a 10-membered hydrogen-bonded ring between the amide carbonyl of residue i and the amide proton of residue $i + 3$.[1] There is a great deal of interest in the synthesis of small molecules that mimic the β-turn structure, for example, to mimic or interfere with protein−protein interactions[2] or bind to biological targets.[3]

Numerous β-turn mimetics have been reported. Many are, however, not generally applicable and cannot contain any desired side chain, if any. Furthermore, the resemblance to a β-turn in solution may be absent.[4] The absence of a reliable solid-phase synthesis route may also be an impediment to the introduction of diversity. Favorable exceptions are described, among others,[3] by Ellman et al.,[5] Kahn et al.,[6] Golebiowski et al.,[7] and Burgess et al.,[8] who have designed β-turn mimicking ring systems which have also been successfully used in combinatorial preparations.

As part of a program directed toward covalent control of shape and folding in peptides, we were interested in developing a general approach for small molecule β-turn mimetics, which was also amenable to combinatorial purposes (Figure 1).

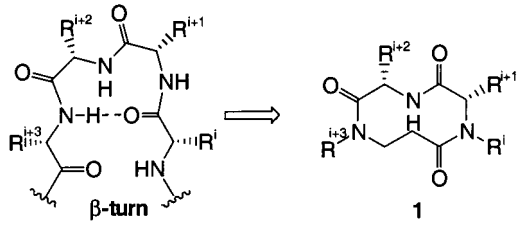


Figure 1. Protein β-turn and β-turn mimetic **1**.

In this Letter we describe the design and synthesis of a potential β-turn mimic of *entirely tripeptidic* nature.[9] A

(1) Ball, J. B.; Hughes, R. A.; Alewood, P. F.; Andrews, P. R. *Tetrahedron* **1993**, *49*, 3467.
(2) Burgess, K. *Acc. Chem. Res.* **2001**, *34*, 826.
(3) For a recent review, see: Souers, A. J.; Ellman, J. A. *Tetrahedron* **2001**, *57*, 7431.
(4) Müller, G.; Hessler, G.; Decornez, H. Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 894.
(5) (a) Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Tetrahedron Lett.* **1996**, *37*, 6961. (b) Souers, A. J.; Virgilio, A. A.; Schürer, S. C.; Ellman, J. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2297. (c) Haskell-Luevano, C.; Rosenquist, Å.; Souers, A.; Khong, K. C.; Ellman, J. A.; Cone, R. D. *J. Med. Chem.* **1999**, *42*, 4380. (d) Souers, A. J.; Virgilio, A. A.; Rosenquist, Å.; Fenuik, W.; Ellman, J. A. *J. Am. Chem. Soc.* **1999**, *121*, 1817.

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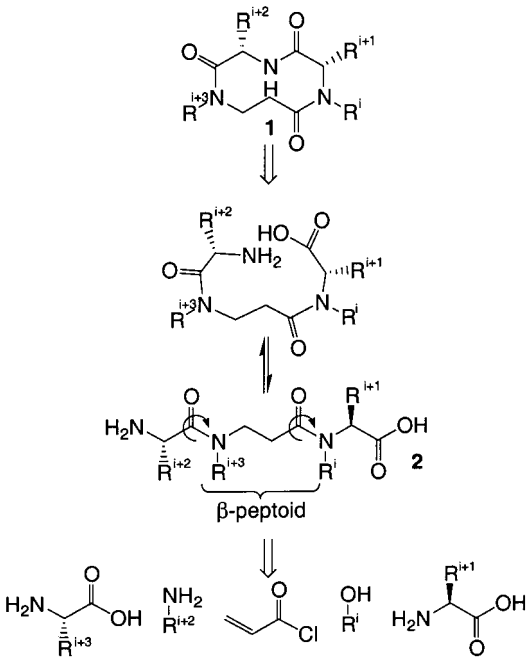
Scheme 1. Retrosynthesis of β-Turn Mimetics with General Structure **1**



corresponding tripeptidomimetic containing an amino sulfonamide will be reported shortly. Examples of cyclic tri- and tetrapeptides consisting only of (L) amino acids are very scarce[10] and usually contain proline, an *N*-substituted amino acid, or the very flexible amino acid glycine, which has no side chain. The presence of proline or an *N*-substituted amino acid is especially noteworthy, since this will facilitate rotation around the amide bond from the *trans*- to the *cis*-rotamer. This rotation is necessary for the nine-membered ring of the cyclic tripeptide to form. Clearly, the presence of only *trans*-rotamers of the peptide amide bond will hamper the formation of a small cyclic peptide.[11]

We designed cyclic ($\alpha_2\beta$)-tripeptides of general structure **1** as mimics of β-turns (Scheme 1). A crucial feature of our

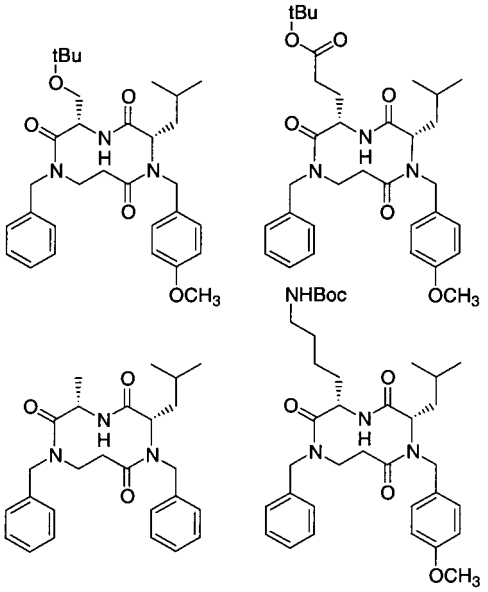


Figure 2. Examples of synthesized cyclic tripeptides.

design was the use of substituted amide bonds in the precursors in order to facilitate rotation and thereby cyclization.[12] The presence of four substituents (R^i−R^{i+3}) in principle provides ample opportunity for the generation of diverse libraries. In **1** the H-bond of the 10-membered ring of a natural β-turn is replaced by a β-peptoid residue,[13] providing covalent control of the structure of the turn as well as facilitating rotation around the amide bond.

The retrosynthesis of **1** is shown in Scheme 1. Our strategy for creating the β-turn mimetics was to leave the central ($i + 1$ and $i + 2$) amino acids of the β-turn largely untouched with respect to a naturally occurring β-turn. The two amide substituents R^i and R^{i+3} were introduced to facilitate rotation around the tertiary peptide amide bonds. Moreover, they provide additional diversity as pharmacophores in the ultimate tripeptide β-turn mimic. Alternatively, one of the substituents may be used as a resin attachment site. Ring closure to **1** should take place in the final coupling step involving two α-amino acid residues, which should give the highest coupling efficiency. Nevertheless, because structure **1** is between a tri- and tetrapeptide in size, and therefore considerably rigid, it was expected that cyclization would be difficult.

Cyclization precursor **2** should be readily accessible using previously described chemistry. The β-peptoid residue in **2** can be assembled through Michael addition of a primary amine to a resin-bound acrylate,[14] while the *N*-alkyl substituent comprising the R^i side chain can be introduced using

(6) (a) Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. *J. Am. Chem. Soc.* **1999**, *121*, 12204. (b) Kim, H.; Nakanishi, H.; Lee, M. S.; Kahn, M. *Org. Lett.* **2000**, *2*, 301. (c) Eguchi, M.; Lee, M. S.; Stasiak, M.; Kahn, M. *Tetrahedron Lett.* **2001**, *42*, 1237.

(7) (a) Golebiowski, A.; Klopfenstein, S. R.; Chen, J. J.; Shao, X. *Tetrahedron Lett.* **2000**, *41*, 4841. (b) Golebiowski, A.; Klopfenstein, S. R.; Shao, X.; Chen, J. J.; Colson, A.; Grieb, A. L.; Russell, A. F. *Org. Lett.* **2000**, *2*, 2615.

(8) (a) Feng, Y.; Burgess, K. *Chem. Eur. J.* **1999**, *5*, 3261. (b) Feng, Y.; Pattarawarapan, M.; Wang, Z.; Burgess, K. *Org. Lett.* **1999**, *1*, 121. (c) Maliartchouk, S.; Feng, Y.; Ivanisevic, L.; Debeir, T.; Cuello, C.; Burgess, K.; Saragovi, H. U. *Mol. Pharmacol.* **2000**, *57*, 385. (d) Zhang, A. J.; Khare, S.; Gokulan, K.; Linthicum, D. S.; Burgess, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 207.

(9) A corresponding tripeptidomimetic containing an amino sulfonamide will be reported shortly.

(10) See, for example: (a) Schmidt, U.; Langner, J. *J. Pept. Res.* **1997**, *49*, 67. (b) Ehrlich, A.; Heyne, H. U.; Winter, R.; Beyermann, M.; Haber, H.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1996**, *61*, 8831. (c) Mästle, W.; Link, U.; Witschel, W.; Thewalt, U.; Weber, T.; Rothe, M. *Biopolymers* **1991**, *31*, 735. (d) Nishino, N.; Xu, M.; Maihara, H.; Fujimoto, T. *Tetrahedron Lett.* **1992**, *33*, 1479.

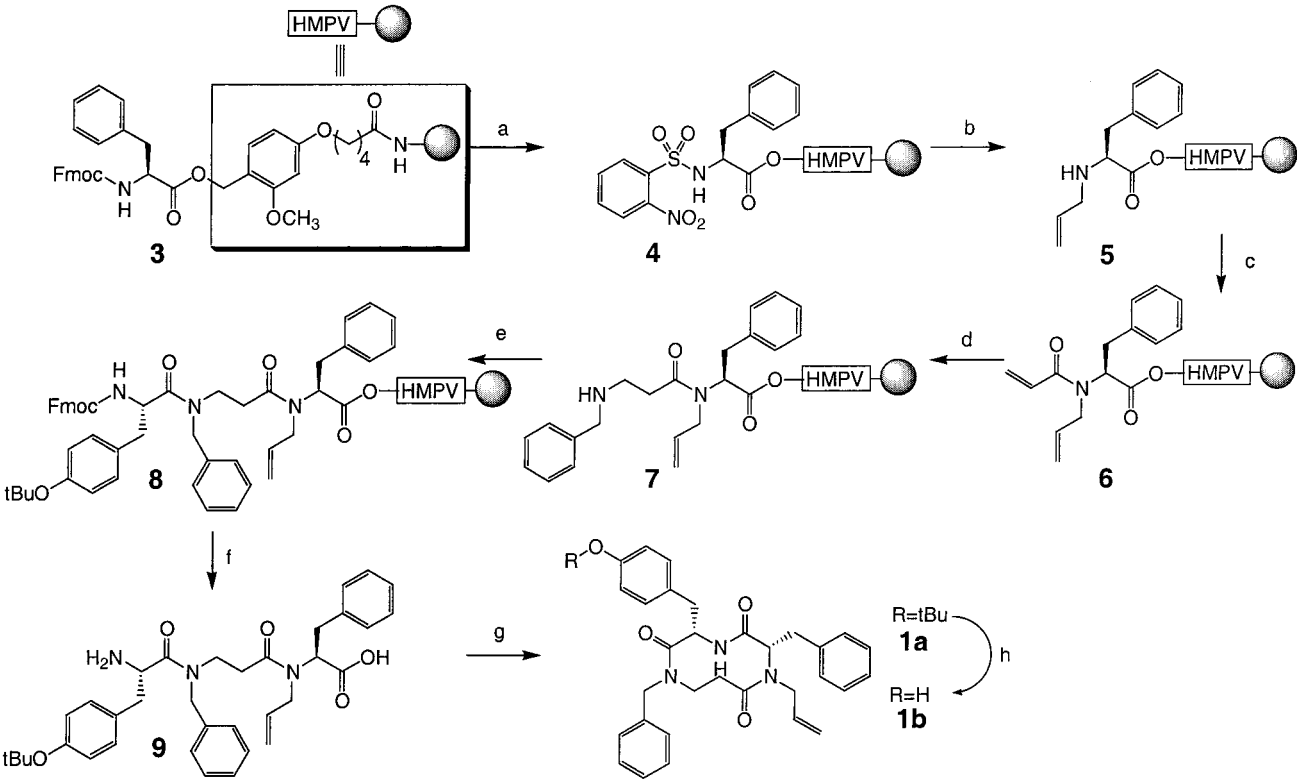
(11) Scherer, G.; Kramer, M. L.; Schutkowski.; M. Reimer, U.; Fischer, G. *J. Am. Chem. Soc.* **1998**, *120*, 5568.

(12) The favorable influence on cyclization by an N-substituted amide containing a Boc-group was described in: Cavalier-Frontin, F.; Achmad, S.; Verducci, J.; Jacquier, R.; Pèpe, G. *J. Mol. Struct. (THEOCHEM)* **1993**, *286*, 125.

(13) Hamper, B. C.; Kolodziej, S. A.; Scates, A. M.; Smith, R. G.; Cortez, E. *J. Org. Chem.* **1998**, *63*, 708.

(14) Brown, A. R.; Rees, D. C.; Rankovic, Z.; Morphy, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 3288

Scheme 2. Synthesis of a Representative Mimetic[a]



[a] Reagents and conditions: (a) (i) 20% piperidine, DMF, 30 min; (ii) oNBS-Cl (5 equiv), collidine (10 equiv), 1 h; (b) (i) allyl alcohol (10 equiv), Ph_3P (5 equiv), DIAD (5 equiv), DCE, 1 h; (ii) 0.5 M β-mercaptoethanol, DBU (5 equiv), 30 min; (c) acryloyl chloride (4 equiv), TEA (6 equiv), 16 h; (d) benzylamine (20 equiv), LiCl, DMSO, 3 days; (e) Fmoc-Tyr(tBu)-OH (4 equiv), HBTU (4 equiv), HOAt (0.5 equiv), collidine (8 equiv), 16 h; (f) (i) 20% piperidine, DMF, 30 min; (ii) 1% TFA/DCM, 10 × 2 min; (g) BOP (1.1 equiv), HOBt (1.1 equiv), DIEA (2.5 equiv), 16 h; (h) TFA, 30 min.

our "site-specific *N*-alkylation" method.[15] Thus, it should ultimately be possible to introduce a large amount of diversity, using an array of alcohols for the R^i side chains and amines for the R^{i+3} side chains. This diversity can be further increased by accessing the large pool of commercially available α-amino acids for the R^{i+1} and R^{i+2} side chains.

As an illustration of its potential, the synthesis of a representative (**1b**) of this new class of potential turn mimetics is shown in Scheme 2. The synthesis of **1b** commenced with resin-bound Fmoc-phenylalanine **3**, attached to the resin through the highly acid labile HMPV (4-(4-hydroxymethyl-3-methoxyphenoxy)-valeric acid) linker.[16] After Fmoc deprotection, a solid-supported sulfonamide (**4**) having a relatively acidic proton was generated using oNBS-Cl.[17] This sulfonamide was suitable for introduction of an *N*-allyl substituent by a Mitsunobu reaction on the solid phase. Next, the oNBS group was removed using a thiolate anion, affording the resin-bound deprotected secondary amine **5**, which was reacted with acryloyl chloride. The resulting resin-bound acrylate **6** was then subjected to a Michael addition yielding secondary amine **7**, to which Fmoc-Tyr-(tBu)-OH was coupled to give **8**. After cleavage of the Fmoc group, the linear αβα ($\alpha_2\beta$) tripeptide could be selectively released from the resin by mild acid treatment, leaving the side chain protection intact. After purification, linear trimer **9** was obtained in 68% yield (average yield 96% per step, 9 steps). Cyclization of **9** was achieved at 1 mM concentration in DCM using BOP leading to ($\alpha_2\beta$)-tripeptide **1a** in 55% yield. Because cyclization is relatively slow, the activated C-terminal residue might have partly racemized.[18] Efforts are underway to clarify the issue of racemization. Finally, removal of the side chain tBu protecting group was accomplished using TFA to yield the desired cyclic ($\alpha_2\beta$-) tripeptide **1b** in 11% yield after preparative HPLC. Yields in this last deprotection step turned out to be somewhat disappointing because of the observed moderate acid stability of the resulting cyclic tripeptides. Instability of α-amino acid containing cyclic tripeptides has been noted before.[19] De-

(15) (a) Reichwein, J. F.; Liskamp, R. M. J. *Tetrahedron Lett.* **1998**, *39*, 1243. (b) Reichwein, J. F.; Wels, B.; Kruijtzer, J. A. W.; Versluis, C.; Liskamp, R. M. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 3684.

(16) The HMPV linker is analogous to the HMPB linker introduced by Riniker et al. and is available from Senn Chemicals AG. Riniker, B.; Flörsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307.

(17) Fukayama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.

(18) The NMR spectra show additional peaks that may be due to the presence of multiple conformations of the cyclic trimer or may be caused by the presence of the epimeric product.

(19) (a) Rothe, M.; Faehnle, M.; Wermuth, S. *Pept., Proc. Eur. Pept. Symp., 18th* **1984**, 573. (b) Rothe, M.; Faehnle, M.; Maestle, W.; Feige, K. *Pept.: Struct. Funct., Proc. Am. Pept. Symp., 9th* **1985**, 177.

composition is complete within 2 h upon treatment with 10% TFA in DCM, so it is essential that the deprotection time is kept to a minimum. However, this problem can be addressed by adapting the protecting groups used.

Using this route we were able to synthesize a small array of other (functionalized) cyclic ($\alpha_2\beta$) tripeptides in similar yields (Figure 2). Since this method is clearly amenable to combinatorial approaches, work is in progress on the synthesis of libraries of these potential small molecular β-turn mimetics. Indeed, preliminary modeling studies using Monte Carlo conformational searching implemented in the MacroModel[20] program (vs 7.0) showed a clear resemblance of the global minimum of the $\alpha_2\beta$-tripeptide backbone with a type III′ turn (Figure 3).[1] The R^i-R^{i+1} distance was 6.8 Å, nicely fitting within the 7 Å limit.[1]

In conclusion, we have shown the synthesis of putative small molecule β-turn mimetics comprising a cyclic ($\alpha_2\beta$) tripeptide skeleton starting from simple starting materials of which a large diversity is available. Using this route a variety of functionalized cyclic tripeptides was accessible. Moreover, to our knowledge, these are the smallest readily functionalized cyclic peptide systems that have been described so far. A crucial feature of our approach is facilitation of rotation around amide bonds by using substituted, i.e., tertiary amide bonds, thereby enabling cyclization. Currently, on-resin cyclization methods are under investigation. In addition, modifications of the β-peptoid moiety are being investigated in order to further expand the diversity of the cyclic tripeptide skeleton to tripeptidomimetics.

(20) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

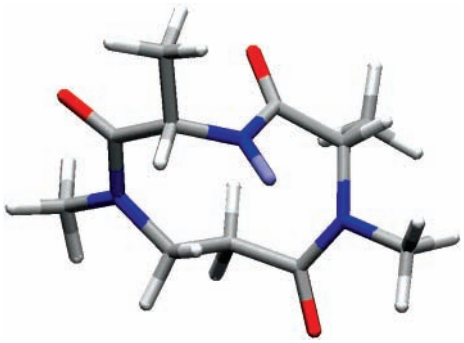


Figure 3. Global minimum of the $\alpha_2\beta$-tripeptide backbone. The calculated torsion angles were $\Phi^{i+1} = 59°$, $\psi^{i+1} = 44°$, $\Phi^{i+2} = 66°$, $\psi^{i+2} = 30°$.

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Supporting Information Available: Experimental procedures and characterization details (HPLC, ^{1}H, ^{13}C, COSY and TOCSY spectra) of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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