Simultaneous Solid-Phase Synthesis of β-Turn Mimetics Incorporating Side-Chain Functionality

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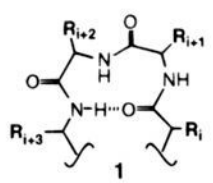
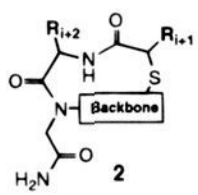
Received July 15, 1994

β-Turns, **1**, are one of the three major secondary structural elements of peptides and proteins and play a key role in many of the molecular recognition events in biological systems including the interaction between peptide hormones and their receptors, antibodies and antigens, and regulatory enzymes and their corresponding substrates.[1] A great deal of effort, therefore, has been focused on the design of small constrained mimetics of turn structure in order to provide a better understanding of the molecular basis of peptide and protein interactions in addition to providing potent and specific therapeutic agents.[2] These efforts, however, have met with only limited success due to difficulties in identifying the key turn residues and the relative orientations of those residues in the receptor-bound conformation. This is compounded by the fact that efficient methods for constructing turn mimetics incorporating both the $i + 1$ and $i + 2$ side chains have not yet been developed. The aforementioned difficulties in identifying the optimal structure for a given turn mimetic could be circumvented by the synthesis and screening of a combinatorial library of β-turn mimetics which include all possible side-chain combinations as well as multiple relative orientations of the side chains. Herein we report a general and expedient method for the solid-phase synthesis of β-turn mimetics that incorporate a variety of side-chain functionality and demonstrate the simultaneous synthesis of multiple turn derivatives.



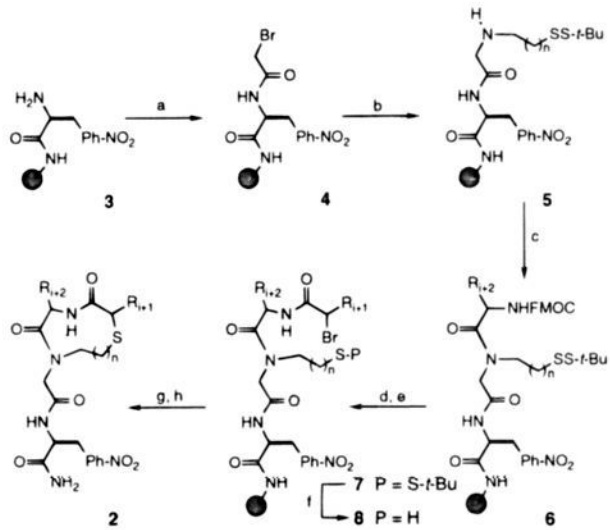


The turn mimetic **2** is constructed from three readily available components. The $i + 1$ side chain is derived from an α-halo acid, and the $i + 2$ side chain is derived from an α-amino acid. The mimetic is constrained in a turn structure by replacing the hydrogen bond between the i and $i + 3$ residues with a covalent backbone linkage. The flexibility of the turn mimetic as well as the relative orientations of the side chains can be varied by introducing different backbone linkages, for example, to provide 9- or 10-membered rings, as well as by preparing different combinations of the absolute configurations at each of the stereocenters introduced by the $i + 1$ and $i + 2$ side chains of the turn mimetic.

Before the construction of turn mimetic **2** was initiated (Scheme 1), *p*-nitrophenylalanine was coupled to the (4-((2′,4′-dimethoxyphenyl)aminomethyl)phenoxy)acetic acid derivatized resin[3] employing standard solid-phase peptide synthesis methods to provide precursor **3**. Upon cleavage of the final turn mimetic from the support, the *p*-nitrophenylalanine chromophore serves as a convenient UV tag for accurate determination of the overall purity of the turn mimetic (vide infra). α-Bromoacetic acid is first coupled to the support-bound *p*-nitrophenylalanine by activation with diisopropylcarbodiimide. The backbone element is then introduced by treatment of α-bromo amide **4** with either 2-aminoethanethiol *tert*-butyl disulfide or 3-aminopropanethiol *tert*-butyl disulfide in DMSO to provide the secondary amine **5**,[4] which is then coupled with the appropriate FMOC-protected amino acid employing *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) to provide **6**.[5] Treatment with 20% piperidine in DMF followed by reaction with the symmetric anhydride of the appropriate α-bromo acid provides acyclic intermediate **7**, which incorporates both the $i + 1$ and the $i + 2$ side-chain residues. It is essential that the symmetric anhydride of the α-bromo acid be employed in the coupling step, since direct activation of the α-bromo acid with 1 equiv of carbodiimide results in significant racemization.[6] Cleavage of the mixed disulfide is then accomplished by treatment with tributylphosphine in a 5:3:2 propanol/DMF/water comixture to provide cyclization precursor **8**. It should be noted that, for clean reduction of the disulfide bond without side reactions, it is necessary to employ a polyethylene glycol−polystyrene graft copolymer (PEG−PS)[7] as the solid support rather than macroreticular or gel forms of polystyrene, due to the inability of these resins to be solvated by the aqueous solvent comixture.

Rapid cyclization to provide the 9- or 10-membered thioether is accomplished by treatment with tetramethylguanidine in a propanol/DMF/H_2O comixture.[8] Reaction completion can readily be determined via the Ellman test for free thiols.[9] Cleavage of the turn mimetic from the support is then ac-

Scheme 1[a]



[a] α-Bromo acid, DICI; (b) 2-aminoethanethiol *t*-butyl disulfide or 3-aminopropanethiol *tert*-butyl disulfide; (c) *N*-FMOC-α-amino acid, HATU; (d) 20% piperidine in DMF; (e) symmetric anhydride of α-bromo acid; (f) tributylphosphine, H_2O; (g) tetramethylguanidine; (h) 1:1:18 H_2O/Me_2S/trifluoroacetic acid.

(1) For a comprehensive review of β-turns in peptides and proteins, see: Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1−109.

(2) For reviews on β-turn mimetics, see: (a) Ball, J. B.; Alewood, P. F. *J. Mol. Recognit.* **1990**, *3*, 55−64. (b) Kahn, M., Guest Ed. *Tetrahedron* **1993**, *49*, Symposia 50, 3433−3677.

(3) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787−3790.

(4) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.

(5) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(6) Previous researchers have reported difficulties in coupling α-halo acids employing the standard additive hydroxybenzotriazole. Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646−10647.

(7) Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 113−129. The polyethylene glycol−polystyrene graft copolymer can be purchased from Rapp Polymere, Tubingen, Germany.

(8) Researchers at Genentech have reported the synthesis of 15-membered ring RGD mimetics by thioalkylation. Barker, P. L.; et al. *J. Med. Chem.* **1992**, *35*, 2040−2048.

(9) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.

0002-7863/94/1516-11580$04.50/0

Table 1. β-Turn Mimetics **2** (Scheme 1)

	derivative[a]			purity (%)[b]	
2	R_{i+1}	R_{i+2}	backbone (*n*)	PEG-PS	pins
a	CH_3	CH_2Ph	2	90	79
b	CH_3[c]	CH_2Ph	2	59	90
c	$CH(CH_3)_2$	CH_2Ph	2	81	86
d	CH_2CO_2H	CH_2Ph	2	65	79
e	CH_3	$(CH_2)_4NH_2$	2	72	87
f	CH_3	CH_2CO_2H	2	63	86
g	H	CH_2Ph	2	85	91
h	H	CH_2OH	2	82	93
i	CH_3	$CH_2C_6H_4$-4-OH	2	74	88
j	CH_3	CH_2Ph	1	77	75
k	$CH_2C_6H_4$-4-OH	CH_3	1	81	90

[a] The stereochemical configuration at the $i + 1$ site is *R* and at the $i + 2$ site is *S* unless otherwise specified. [b] Percent purity is determined as the ratio of the peak area of the desired product to the total peak area of all of the products as evaluated by HPLC analysis employing a C18 reverse-phase column with a gradient of 20−100% methanol in 0.1% trifluoroacetic acid in H_2O with monitoring at 270 nm. [c] The stereocenter has the *S* configuration.

complished by treatment of the support with a 1:1:18 water/dimethyl sulfide/trifluoroacetic acid mixture to provide mimetic **2**. As shown in Table 1, the turn mimetics **2** were obtained with a purity of 75% on average over the eight-step process as determined by HPLC analysis.[10] It should be noted that any side products that were produced during the synthesis of the turn mimetic would be detected by HPLC analysis since the UV tag, *p*-nitrophenylalanine, was introduced before the synthesis of the mimetic was initiated.[11]

For all of the turn mimetics synthesized, cyclization provided the desired cyclic monomer with no cyclic dimer detected (<5%).[12] This includes mimetics incorporating both *R* and *S* α-bromo acids, α-bromoisovaleric acid, which corresponds to the sterically hindered amino acid, valine, and α-chloroacetic acid, which corresponds to the least sterically hindered amino acid, glycine, at the $i + 1$ site.[13] Furthermore, as shown in Table 1, a variety of side-chain functionality could be incorporated successfully into the turn mimetics including alcohol, phenol, carboxylic acid, and amine functionality. Racemization during the synthesis of the turn mimetic was evaluated by HPLC analysis of the diastereomeric turn mimetics **2a** and **2b**, which incorporate (*S*)- and (*R*)-α-bromopropionic acid, respectively. In the synthesis of **2a**, less than 5% of the epimer resulting from racemization was observed, while in the synthesis of **2b**, 8% of the epimer resulting from racemization was observed.[14]

To demonstrate the utility of the synthesis sequence for the rapid construction of a library of turn mimetics **2**, the 11 mimetics were synthesized simultaneously by employing a Mimotopes pin apparatus developed for the simultaneous synthesis of multiple peptide derivatives.[15] In the procedure using this apparatus, polyethylene−poly(*N*,*N*-dimethylacrylamide/methacrylic acid) graft copolymer pins that are prederivatized with the acid-cleavable Rink linker are configured such that each pin fits into a well of a 96-well microtiter plate. Even though each microtiter well serves as a unique reaction vessel, the simultaneous synthesis of hundreds to thousands of derivatives can rapidly be accomplished employing microtiter plate-based instrumentation. As shown in Table 1, the simultaneous synthesis of the β-turn mimetics **2** in pin format, according to the reaction conditions described above, provided all 11 derivatives in a very high level of purity as determined by HPLC analysis, thus demonstrating the feasibility of rapidly constructing a β-turn mimetic library.

A 9-membered β-turn mimetic lacking the *p*-nitrophenylalanine chromophore and incorporating methyl side chains at both the $i + 1$ and $i + 2$ sites in the *R* and *S* configurations, respectively, was chosen as a model compound with which to investigate the conformations of mimetics **2** and to evaluate the ability of these compounds to mimic actual β-turns. The ^1H NMR spectrum of the model compound in 10% DMSO-d_6 in water at room temperature revealed one conformer. The 9.5 Hz coupling constant between the central amide N−H and the Cα−H of the $i + 2$ residue was used to constrain the backbone dihedral angle (ϕ_{i+2}) to $-120° \pm 20°$ for a Monte Carlo conformational search analysis using Amber* force field and GB/SA solvation as implemented in MacroModel.[16] The lowest energy conformation (20 kJ more stable than the next lower conformer) was compared to ideal β-turns of types I, I′, II, II′, III, and III′ by calculation of a least-squares fit between the S, Cα(i+1), Cα(i+2), and C(i+2) atoms of the mimetic and the corresponding N(i+1), Cα(i+1), Cα(i+2), and C(i+2) atoms of the β-turn.[17] On the basis of this comparison, the lowest energy conformer was found to best fit a type II′ turn (rms = 0.29 Å).[18] The conformations of a number of turn mimetics of different ring sizes and stereochemical configurations at the $i + 1$ and $i + 2$ sites are under further investigation. The construction and biological evaluation of a large library of turn mimetics **2** is also in progress and will be reported in due course.

Acknowledgment. We gratefully acknowledge the NSF, the Beckman Foundation, the Burroughs Wellcome Fund, the A. P. Sloan Foundation, Affymax, Tularik, and Chiron for support of this work. A.A.V. also thanks Berlex for a graduate fellowship. We would also like to thank Andrea Sefler, George Lauri, and Paul Bartlett for providing invaluable assistance with the conformational analysis of the model compound.

Supplementary Material Available: Experimental details for the synthesis and conformational analysis of the β-turn mimetics (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) All β-turn mimetics were characterized by NMR and HRMS (FAB) analysis.

(11) Due to the mechanical instability of the solid support, it is essential that the reaction mixture be agitated by rocking or an N_2 flush, since vigorous stirring or shaking resulted in considerable loss of material from the solid support.

(12) Cyclization to provide **2** was initially attempted by macrolactamization between the $i + 1$ and $i + 2$ residues rather than by thioalkylation; however, cyclization with a range of activating agents and solid supports provided significant amounts of cyclic dimer. These results are in accord with the well-precedented difficulties in macrolactamization to provide 9- and 10-membered-ring structures. (a) Story, S. C.; Aldrich, J. V. *Int. J. Pept. Protein Res.* **1994**, *43*, 292−296. (b) Kemp, D. S.; Stites, W. E. *Tetrahedron Lett.* **1988**, *29*, 5057−5060.

(13) Introduction of bromoacetic acid at the $i + 1$ position rather than chloroacetic acid results in a dramatically lower yield of desired product.

(14) Less than 5% racemization was observed in the synthesis of **2b** on pins (vide infra).

(15) Maeji, N. J.; Valerio, R. M.; Bray, A. M.; Campbell, R. A.; Geysen, H. M.; *Reactive Polymers* **1994**, *22*, 203−212. The FMOC-RinkAmide-Handle-Gly-HMD-MA/DMA pins (7.3 μmol/pin) were supplied by Chiron Mimotopes (Victoria, Australia).

(16) MacroModel V4.5: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(17) A similar choice of atoms was made by Olson to match a 9-membered β-turn mimetic to a type II′ turn (rms = 0.31 Å). Olson, G. L.; et al. *J. Am Chem. Soc.* **1990**, *112*, 323−333.

(18) A good fit was also found to a type I′ turn (rms = 0.23 Å) although the central amide bond was flipped.