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Expedient Solid-Phase Synthesis of Putative β-Turn Mimetics Incorporating the i + 1, i + 2, and i + 3 Sidechains

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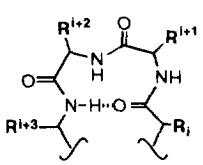
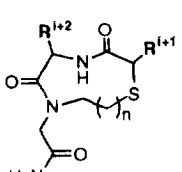
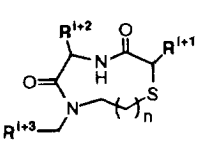
Abstract: A number of putative β-turn mimetics that incorporate sidechain functionality at the i + 1, i + 2, and i + 3 positions were synthesized on solid support in high levels of purity and in good overall yields from readily available starting materials. A variety of sidechain functionality, ring sizes, and sidechain stereochemistries were introduced demonstrating the generality of the synthesis method. A significant feature of the synthesis sequence is the use of a guanidine resin that functions as both a solid-phase extraction resin and a support-bound general base catalyst.

Much effort has been devoted to the design and synthesis of general peptidomimetic scaffolds modeled on elements of peptide and protein secondary structure. The β-turn structure has received a great deal of attention due to its ubiquitous nature, and a number of β-turn mimetics have been reported.[1] In order to attain high-affinity and selective binding to a targeted receptor, the compound must mimic both the sidechain functionality and the three-dimensional display of that functionality,[2] which is complicated by difficulties in identifying the key sidechain residues and the relative orientations of these residues in the receptor-bound conformation. The synthesis and biological evaluation of arrays of β-turn mimetics that contain a number of sidechain combinations as well as different relative orientations of these sidechains would provide invaluable insight into both the functional and structural requirements essential for high binding affinity and/or specificity.

Earlier we reported the solid-phase synthesis of mimetics **1** incorporating sidechain functionality at the i + 1 and i + 2 positions.[3] We have since employed this methodology in the generation of a diverse library of 1152 turn mimetics from which moderate affinity f-Met-Leu-Phe antagonists have been identified.[4] Here we report the synthesis of second generation mimetics **2** designed to possess improved binding affinity, solubility, and perhaps bioavailability,[5] by incorporating the i + 3 sidechain and by eliminating the primary amide functionality. Synthesis of mimetics **2** involves two major modifications. First, sidechain functionality at the i + 3 position is introduced by means of a primary amine. Second, attachment to the solid support during the solid phase synthesis sequence is accomplished using a disulfide linkage rather than the Rink amide linkage[6] that was employed in the synthesis of **1**. When the final cyclic product **2** is formed, no vestiges of the linkage to the support remain.



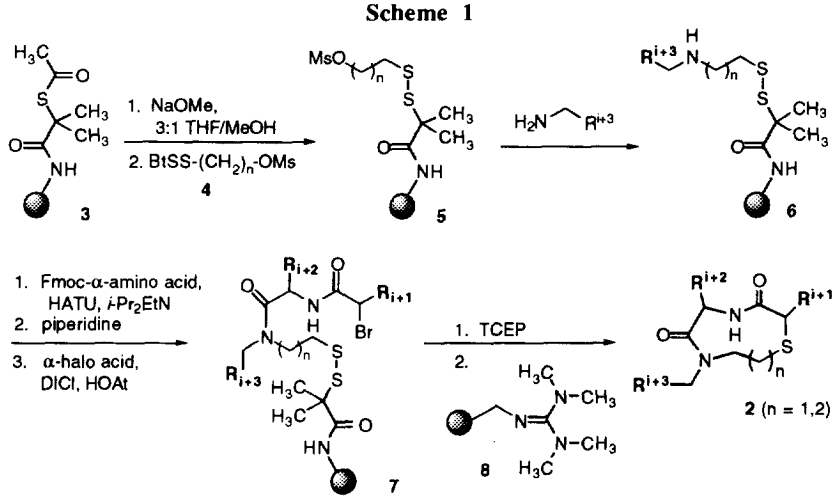




The second generation mimetic presents a scaffold that is amenable to the rapid and simultaneous synthesis of a number of derivatives. Mimetic **2** is assembled from four components in eight steps on a solid support. An α-halo acid supplies the i + 1 sidechain, a Fmoc-protected α-amino acid supplies the i + 2 sidechain, a primary amine supplies the i + 3 sidechain, and a hydroxyalkyl thiol provides the backbone component.

Linker **3** which is derived from *S*-acetyl 2-mercapto-2-methyl propionic acid is employed to tether the second generation turn mimetic to the solid support through the hydroxyalkyl thiol backbone component.[7] The *gem*-dimethyl substituents adjacent to the sulfur atom are necessary to improve the stability of the eventual disulfide bond. Absence of the *gem*-dimethyl substituents, such as with the corresponding 2-mercaptoacetic acid derived linker, results in reduced yields presumably due to lability of the disulfide bond to amine bases. Methanolysis of thioester **3** followed by disulfide interchange with the methanesulfonoxyalkyl thiol backbone component activated as the 2-benzothiazolyl (Bt) mixed disulfide **4**[8] provides mesyl ester **5**. Completion of the disulfide interchange is monitored by the disappearance of free thiol on the support.[9] Although the backbone component can also be loaded onto the support as the free alcohol followed by activation by mesylation with mesyl anhydride and 2,6-lutidine in CH_2Cl_2, slightly lower yields of the final mimetics are observed.

Scheme 1

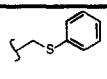
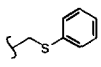
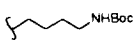
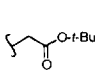
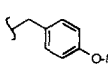
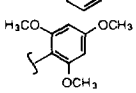
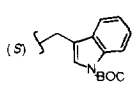
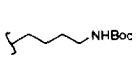
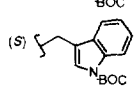
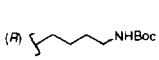


The support-bound mesylate **5** is treated with a concentrated solution of the appropriate primary amine to introduce the i + 3 sidechain to give **6**. The i + 2 and i + 1 sidechains are then incorporated in a manner similar to that described for the first generation of turn mimetics. A Fmoc-protected amino acid is coupled to secondary amine **6** using O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexa-fluorophosphate (HATU). The Fmoc protecting group is removed and the free amine is acylated with an α-halo acid to give **7**. The acyclic turn mimetic **7** is liberated from the solid support by treatment with tris-(2-carboxyethyl)phosphine (TCEP).[10] The acyclic turn mimetic is then treated with support-bound guanidine **8**.[11] The support-bound guanidine functions as both a solid-phase extraction resin and a support-bound catalyst; it removes TCEP oxide, excess TCEP, and the byproduct, HBr, from solution, and acts as a general base to promote thioether formation to yield cyclic product **2**. Cyclization proceeds to completion within 24 h at room temperature. Furthermore, the formation of cyclic dimer, oligomer, disulfide, or other product resulting from the interaction of two or more acyclic compounds is not observed when the cyclization reaction is performed at 1 mM substrate concentrations. Filtration to remove the resin, followed by concentration in vacuo and flash chromatography gives mimetic **2**. Compounds containing sidechain protecting groups may be deprotected under standard conditions by treatment with 18:1:1 trifluoroacetic acid/dimethyl sulfide/water.[12]

Eight mimetics **2a-h** are included in Table 1 to demonstrate the generality of the synthetic sequence.[13] High levels of purity (>90%) as determined by HPLC and ^{1}H NMR were observed for each of the crude

mimetics **2** obtained directly from the resin. In addition, the average overall yield for the chromatographed and analytically pure material was 55% based upon the expected loading of **3**. The only identifiable side-products (≤ 5%) were the minor diastereomers corresponding to inversion about the α-carbon at the i + 1 position. This most likely results from the initial optical purity of the α-bromo acids that supply the i + 1 sidechain (generally ~95% ee as determined by chiral G.C. analysis), although a small amount of racemization during the synthesis sequence cannot be ruled out. The successful introduction of the functionalized lysine, aspartic acid, tyrosine, and tryptophan sidechains, 9- and 10- membered ring sizes, and both stereochemistries at the i + 1 and i + 2 sites clearly demonstrates the broad generality of the synthesis sequence.

Table 1. Synthesis of Mimetics **2**.[a]

Entry	R^{i+1}	R^{i+2}	R^{i+3}	n	Yield (%)[b]	Purity (%)[c]
2a				1	60	92
2b				1	64	94
2c				1	55	86
2d				1	61	93
2e				2	34	85
2f				2	51	94
2g				1	59	84
2h				1	59	93

[a] The stereochemical configuration of the α-carbon at the i + 1 site is *R* and at the i + 2 site is *S* unless otherwise specified. [b]Mass balance yields of purified, analytically pure material are based upon the approximate loading level of **3** as determined by exhaustively acylating the same batch of aminomethylated resin with *N*-Fmoc-α-aminoisobutyric acid, employing PyBOP and HOBt, until a negative bromophenol blue test is observed, followed by Fmoc quantitation. [c]The purity of the crude product directly off of the support is determined by reverse phase HPLC, 60-100% CH_3OH in 0.1% aqueous TFA as monitored at 220, 254, or 280 nM.

Compounds **2g** and **2h**, which incorporate the tryptophan and lysine sidechains at the i + 1 and i + 2 positions, respectively, were designed to mimic a putative β-turn region of the peptide hormone somatostatin that has been implicated as critical for receptor binding. The observed binding affinity and selectivity to distinct somatostatin receptor subtypes for a collection of mimetics **2** that contain tryptophan and lysine sidechains will be reported shortly.

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11. Treatment of Merrifield resin with a 1.0 M solution of 1,1,3,3-tetramethylguanidine in CH_2Cl_2 with added Bu_4NI for 12 h at reflux provides resin **8**.
12. The general synthesis sequence follows: Aminomethyl derivatized polystyrene resin is solvated in DMF (10 mL/g of resin), and *S*-acetyl 2-mercapto-2-methyl propionic acid (0.2 M), PyBOP (0.2 M), HOBt (0.2 M), and *i*-Pr_2EtN (0.4 M) are added. After 8 h, the solution is drained and the resin is rinsed with DMF (3x), CH_2Cl_2 (4x), and MeOH (2x), then dried *in vacuo*. Resin **3** is then solvated in a 3:1 THF/MeOH comixture (10 mL/g of resin) and purged with Ar (20 min). NaOMe is added to reach a final concentration of 0.2 M, and the reaction vessel is stoppered and shaken for 1 h. Excess AcOH is added to quench the reaction, and the resin is isolated by filtration. The resin is rinsed with 3:1 THF/MeOH (2x) and CH_2Cl_2 (3x). A 0.1 M solution of the benzothiazolyl-activated disulfide mesylate **4** in CH_2Cl_2 is added to the resin, and the reaction vessel is stoppered under an Ar atmosphere. After 12 h, the resin is isolated by filtration and rinsed with CH_2Cl_2 (5x) and MeOH (2x) to afford **5**. The mesylate **5** is treated with a 1.0 M solution of the desired amine in NMP for 16 h at 50 °C. The resin is isolated by filtration and rinsed with DMF (3x), CH_2Cl_2 (5x), and MeOH (2x) to give **6**. Resin **6** is solvated in DMF (10 mL/g of resin) and the desired Fmoc-protected amino acid (0.2 M), HATU (0.2 M), and *i*-Pr_2EtN (0.4 M) are added. After 8 h, the resin is collected by filtration and rinsed with DMF (3x), CH_2Cl_2 (5x), and MeOH (2x). A solution of piperidine (20% v/v) in DMF is used to deprotect the resin (20 min), and it is rinsed as before. The deprotected resin is solvated in DMF (10 mL/g of resin) and the desired α-halo acid, HOAt (0.1 M), and DICI (0.1 M) are added. After 4 h, the resin is isolated by filtration and rinsed as above to provide **7**. The disulfide linkage of **7** to the solid support is reduced by treatment with a 4.0 mM solution of TCEP in a 9:1 dioxane/H_2O solution (1 mL/μmmol of resin bound **7**), which has been purged with Ar (20 min). After 8 h, the solution of acyclic turn mimetic is transferred via a filtration cannula and under Ar pressure to a flask containing ~30 equiv of support-bound guanidine **8**. After the disappearance of all of the acyclic material (<24 h), the solution is filtered to remove the resin and the filtrate is concentrated *in vacuo* to give **2**.
13. All compounds characterized by ^1H NMR and C, H, and N analysis. For a selected characterization: **2a**: ^1H NMR (d_6-DMSO, 300 MHz, 75°C) δ 0.89 (d, 3, J = 6.2), 0.91 (d, 3, J = 5.8), 1.17 (d, 3, J = 6.6), 1.34-1.42 (m, 1), 1.57-1.61 (m, 1), 1.63-1.74 (m, 1), 2.89 (dd, 1, J = 13.2, 7.7), 2.95-3.11 (m, 3), 3.16-3.40 (m, 3), 3.52 (q, 1, J = 7.6),3.58-3.62 (m, 1), 4.48-4.55 (m, 1), 7.15-7.21 (m, 1), 7.28-7.40 (m, 4), 8.49 (d, 1); LRMS (MALDI-TOF): mass calcd for $C_{19}H_{29}N_2O_2S_2$ (MH^+) 381.2, found 381.7. Anal. Calcd for $C_{19}H_{28}N_2O_2S_2$: C, 59.96; H, 7.42; N, 7.36. Found: C, 59.71; H, 7.27; N, 7.17.

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