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Solid supported high-throughput organic synthesis of peptide β-turn mimetics via Petasis reaction/diketopiperazine formation

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Abstract

High-throughput organic synthesis of bicyclic diketopiperazines **1**, β-turn mimetics, is described. Starting from Merrifield resin-bound piperazine-2-carboxylic acid, first two (R^4 and R^5) side chains are introduced via the Petasis reaction and subsequent amide bond formation. Unblocking the α-amino group of piperazine-2-carboxylic acid, Boc-*N*-protected α-amino acid coupling and deprotection followed by cyclative cleavage introduces the remaining R^2 and R^1 side chains. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: solid-phase synthesis; Petasis reaction; peptide β-turn mimetics; combinatorial chemistry; bicyclic heterocyclic compounds.

The interaction of proteins and peptides with macromolecular receptors is fundamental to many biological processes. The successful transfer of information which occurs during these events is highly dependent on the three-dimensional conformation of the proteins. Consequently, much effort has been directed toward the synthesis of metabolically stable, therapeutic peptide equivalents, particularly conformationally restricted reverse turn mimetics.[1]

The β-turn is a common feature in biologically active peptides and is defined as any tetrapeptide sequence with a 10-membered intramolecularly H-bonded ring, in which the $C_\alpha{}^i$ to $C_\alpha{}^{i+3}$ distance varies from 4 to 7 Å. Contingent upon the dihedral angle values for ϕ_2, ψ_2, ϕ_3 and ψ_3 there are at least 14 types of β-turn structures described in the literature.[1b,2]

The overall objective of our high throughput organic synthesis group is to accelerate the discovery of novel therapeutics by expanding the production of chemical libraries in alignment with our existing high-throughput screening. As a part of this program, we studied the synthesis of peptide turn mimetics. The analysis of the β-turn structure led us to the design of bicyclic system **1** (Fig. 1). We envisioned using orthogonally protected, resin bound piperazinic acid **2** as a core fragment

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(Scheme 1).[3] Standard Boc deprotection followed by functionalization of the β-nitrogen atom via the Petasis reaction[4] and subsequent amide bond formation led to incorporation of the R^4 and R^5 substituents. Unblocking of the α-nitrogen, followed by standard Boc-*N*-protected α-amino acid coupling, deprotection and cyclative cleavage,[5] introduced the remaining R^2 and R^1 side chains, leading to bicyclic product **1**. Racemic piperazine-2-carboxylic acid was used as a substrate resulting in the formation of all four possible isomers. Minimal asymmetric induction occurred during the Petasis reaction, and consequently the R^4 substituent is introduced as a ca. 1:1 mixture of epimers.

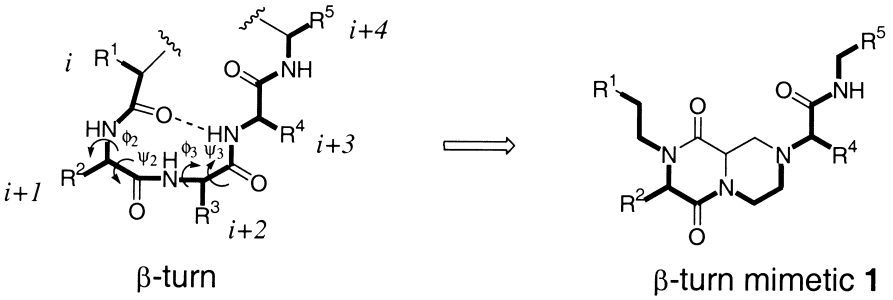
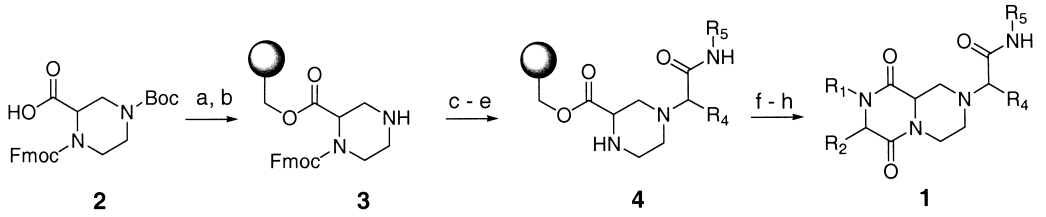


Figure 1.



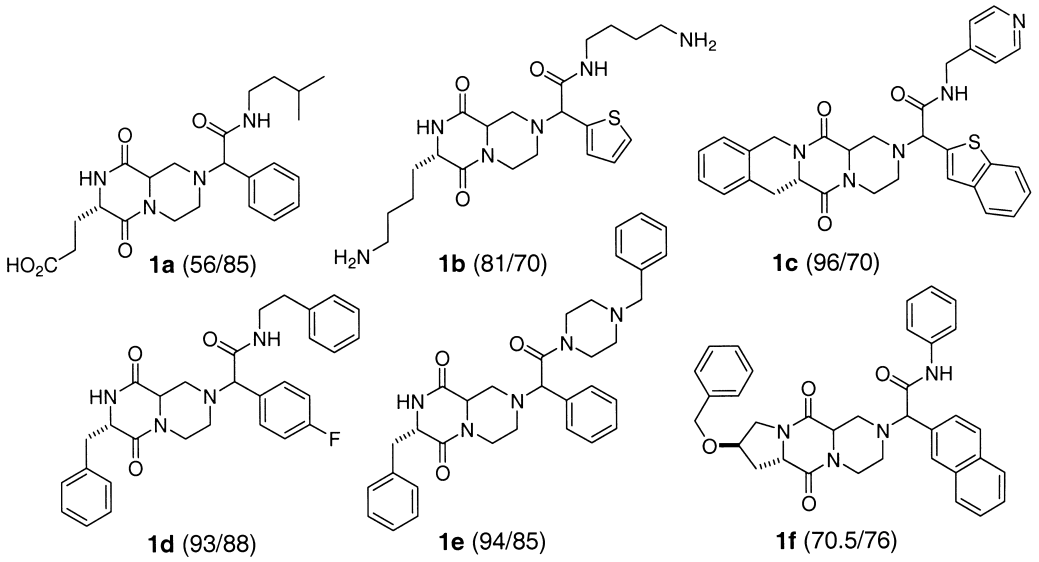
Scheme 1. *Reagents and conditions*: (a) hydroxymethylpolystyrene resin, Ph_3P, DEAD, THF; (b) 40% TFA/DCM, rt, 1 h; (c) $OHCCO_2H{\cdot}H_2O$, R^4-$B(OH)_2$, DCM; (d) DIC, R^5NH_2, DCM; (e) piperidine/DMF 25% sol.; (f) *N*-Boc-α-amino acid, PyBOP, DMF, rt; (g) TFA/DCM 25%, rt, 1 h; (h) 2 M AcOH in *i*-BuOH, 50°C, 24 h

Optimization of the solid phase Petasis reaction[6] and cyclizative cleavage strategy resulted in high purity of most of the products, which could be used directly as crude samples for biological evaluation. The described process is fully amenable to high throughput organic synthesis. Using Robbins FlexChem™ system[7] we were able to rapidly synthesize libraries via the synthetic method described in Scheme 1.[8] The eight-step reaction sequence allows a library to be prepared in 7 days.

The lack of R^3 substituent is an important issue that needs to be addressed. Custom designed 6-substituted-piperazine-2-carboxylic acid analogs could serve as a possible improvement.

Almost all commercially available aryl boronic acids can be used in the above-described protocol (ca. 100). In our experience, boronic acids containing a basic nitrogen atom (e.g. pyridine-4-boronic acid) did not work in this process. Many amines, including anilines (e.g. **1f**, Table 1) can be used (R^5 substituent). Side chains R^1 and R^2 are introduced simultaneously, affording in total a three-dimensional library with a conservatively assessed potential size of $(R^1,R^2)\times R^4\times R^5 = 100\times 1000\times 100 = 10^7$ possible products.

Table 1
Representative products and results. Crude yields (%) and purities (%)(LC/MS with UV detector at 220 nm). The impurities were primarily due to incomplete Petasis reaction



In summary, we have developed a high-throughput organic synthesis protocol for an efficient preparation of bicyclic system **1**. The application of optically pure piperazinic acid would lead to two diastereoisomers. These molecules lack an *i*+3 side-chain substituent, but use of 6-substituted piperazine-2-carboxylic acids would give products that include these pharmacophores. Low costs of all starting materials and product purities in the range of 70–88% make libraries produced by this method very useful for the HTS (high-throughput screening) mapping of peptide turn/receptor interactions. Continued studies on the resolved pure diastereoisomers should reveal a preferred conformation in solution. Relevant results will be reported in due course.

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8. **Typical procedure**: (a) Synthesis of bicyclic diketopiperazine **1a**: Hydroxymethylpolystyrene resin (1.0 g, 1.44 mmol/g, Advanced Chemtech) was swelled in anhydrous dichloromethane (6 mL). Triphenylphosphine (1.13 g, 4.32 mmol) was dissolved in this slurry and the heterogeneous reaction was cooled to 0°C under nitrogen. To this gently stirring solution was added a THF (30 mL) solution of N_{α}-Fmoc-N_{β}-Boc-2-carboxypiperazine **2** (1.95 g, 4.32 mmol) and diethylazodicarboxylate (751 mg, 4.32 mmol) over a period of 30 min. The reaction was allowed to stir for 72 h at which point the resin was filtered and washed with THF (3×), CH_2Cl_2 (3×), MeOH (3×) then multiple, successive and alternating CH_2Cl_2 and MeOH washes (standard manner). (b) Orthogonally protected piperazinic resin ester (ca. 1.3 g) from the previous step was swelled in CH_2Cl_2, filtered and treated with a 40% solution of TFA in CH_2Cl_2 for 1 h. The resin was filtered and washed with CH_2Cl_2 (3×), MeOH (3×), then alternating CH_2Cl_2/MeOH as in (a). The resin bound amine TFA salt was then neutralized with a 10% solution of diisopropylethylamine in CH_2Cl_2 and re-washed in an identical manner as above. (c) The resin ester **3** was swelled in CH_2Cl_2 (mL) and to this was added glyoxylic acid (265 mg, 2.88 mmol) and phenylboronic acid (351 mg, 2.88 mol) as a solution in MeOH:CH_2Cl_2 (20:1). The resulting slurry was agitated for 5 h before filtering the resin and washing with CH_2Cl_2 (3×). The above procedure was repeated again for 16 h after which the resin was again filtered and washed in the standard manner. (d) The resin product from the previous step was swelled in DMF (10 mL) and to this was added HOBt (1.10 g, 7.2 mmol) followed by DIC (1.10 mL, 907 mg, 7.2 mmol). The reaction was agitated for 3 h, after which the resin was washed with DMF (4×). The resin was swelled again in DMF (10 mL) and to this slurry was added *iso*-amyl amine (626 mg, 7.2 mmol) and the reaction was allowed to agitate for 15 h. The resin was filtered and washed with DMF (3×) followed by the standard wash protocol. (e) The resin ester **4** was treated with 25% piperidine in DMF for 45 min. The resin was filtered and washed in the standard manner. The resin was swelled in DMF (20 mL) and to this was added Boc-Glu(O*t*Bu)-OH (2.18 g, 7.2 mmol), PyBOP (3.74 g, 7.2 mmol) followed by diisopropylethylamine (1115 mg, 1.50 mL, 8.64 mmol). The reaction was agitated for 5 h, filtered and rinsed with DMF (3×) before repeating the coupling procedure for 15 h. The resin was filtered and washed in the standard manner. (f) The resin was treated with 25% TFA/CH_2Cl_2 for 1 h. The resin was filtered and washed. The resin was taken up in 10% AcOH/*i*-PrOH and heated for 16 h at 50°C. The resin was filtered off and washed several times with MeOH. The filtrate and washings were combined and concentrated to give an off white solid. The solid was co-evaporated several times with chloroform before being dried under vacuum for 15 h. Yield (282 mg, 56.4%). LC/MS: 85% pure by UV and MS detector. The only other by-product resulted from incomplete deprotection of *t*-butyl ester (glutamic acid side chain) and was easily removed by second TFA treatment (95% TFA/H_2O for 1 h).