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The Development of Hydrazide γ-Turn Mimetics

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Abstract: Monte Carlo calculations show a classical γ-turn in a family of metabolites known as the malformins. This led to the synthesis of epimeric seven-membered ring γ-turn mimetics starting from leucine. NMR temperature coefficient studies were also performed. © 1997 Elsevier Science Ltd.

A rational approach to the design of peptidomimetics is to start with the architectural foundation that is present in nature, namely the folding patterns of the peptide backbone.[1,2] These secondary structures include reverse turns, β-strands, and α-helices. One type of reverse turn consists of three residues and is referred to as a classical γ-turn or C7 conformation. Classical γ-turns are characterized by a 3 → 1 hydrogen bond yielding a pseudo seven-member ring.[3] To complete the peptidomimetic, a conformational constraint is essential to make the turn rigid and reduce the number of degrees of freedom.[4]

Malformins are naturally occurring pentapeptides derived from *Aspergillus niger* (Figure 1).[5] These cyclic peptides were discovered to cause malformations on bean plants, root curvatures on corn plants, and have antibacterial and cytotoxic properties.[6] Sapporo Breweries has reported that these peptides raised the

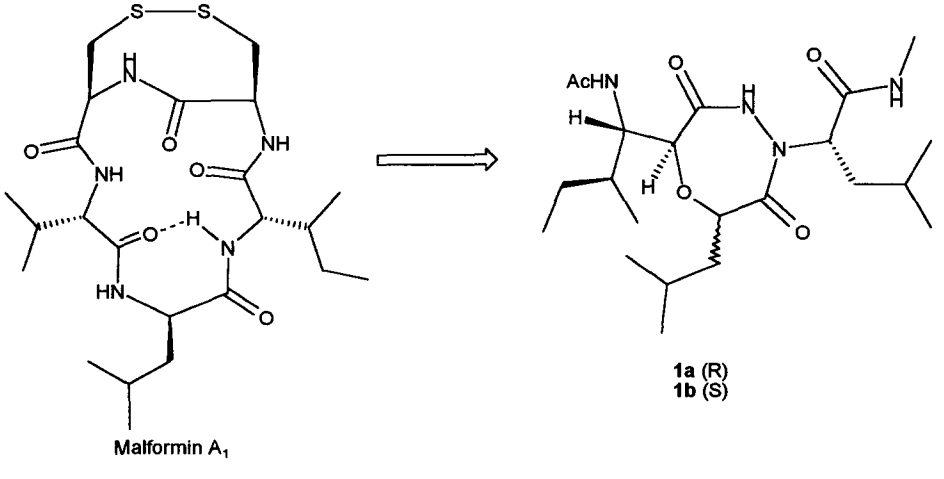
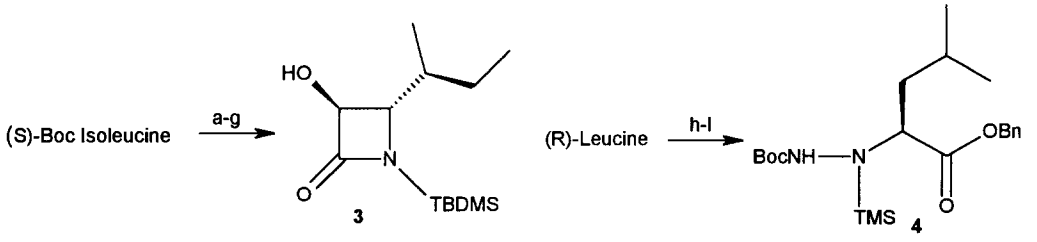


Figure 1

level of Nerve Growth Factor (NGF)[7] 3-6 times in cell culture supernatants at a concentration of 5 μM.[8] Exogenous NGF has been demonstrated to aid degenerating cholinergic neurons and therefore may reverse the chronic symptoms found in diseases such as Alzheimer's disease and ALS.[9]

Monte Carlo conformational analysis of the malformins indicated one dominant ring conformation[10] with an ideal classical γ-turn centered at the D-Leu position and a type I' β-turn at the two D-Cys positions. A seven-membered ring peptidomimetic incorporating the γ-turn portion of the malformins was designed.[11] The syntheses of two γ-turn mimetics (**1a**, **1b**) which are epimeric at the isobutyl group of the ether linkage was planned (Figure 1).

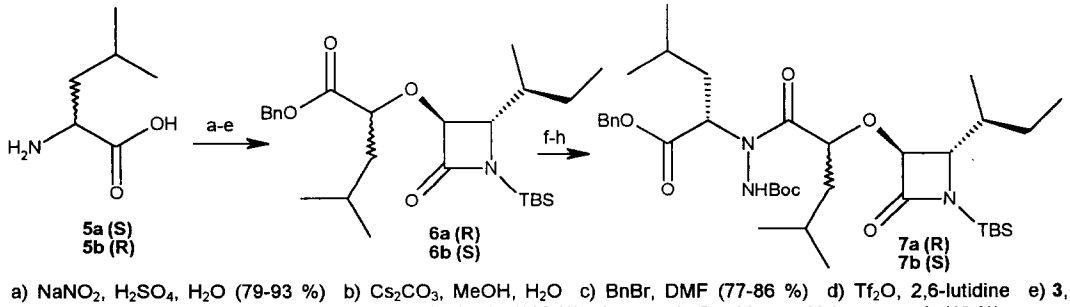
The strategy previously employed in the synthesis of β-turn mimetics was applied to the construction of the γ-turn.[12] Two intermediate pieces were initially synthesized: hydroxy β-lactam **3** and hydrazine **4** (Scheme 1). The salient steps in the synthesis of **4** involved a displacement of the triflate ester with *t*-butyl carbazate[13] followed by activation of the secondary amine upon the addition of *N,O*-bis(trimethylsilyl)amide.[14]



a) IBCF, NMM, Et_2O b) CH_2N_2 (58 %) c) BzOAg, TEA, MeOH (95 %) d) HCl e) TBDMSCl, DMAP, TEA f) *t*-BuMgCl (42 %) g) LDA, oxaziridine (65 %) h) $NaNO_2$, H_2SO_4, H_2O (76 %) i) Cs_2CO_3, MeOH, H_2O j) BnBr, DMF (86 %) k) Tf_2O, 2,6-lutidine, *t*-butyl carbazate, DCM (25 %) l) *N,O*-bis (trimethylsilyl)acetamide

Scheme 1

The synthesis of each epimer of mimetic **1** was achieved from the respective isomer of leucine (**5**) (Scheme 2). Conversion of leucine into the hydroxy acid derivative was followed by protection of the acid as

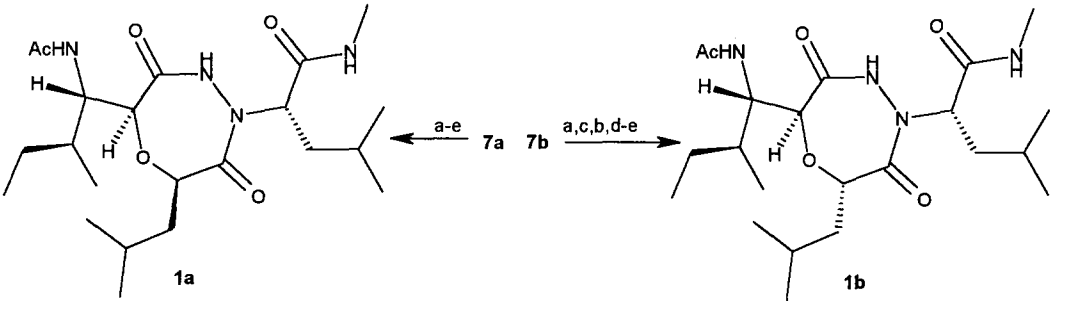


a) $NaNO_2$, H_2SO_4, H_2O (79-93 %) b) Cs_2CO_3, MeOH, H_2O c) BnBr, DMF (77-86 %) d) Tf_2O, 2,6-lutidine e) **3**, NaHMDS, THF, 18-crown-6 (35-44 %) f) H_2, $Pd(OH)_2/C$ (100 %) g) cyanuric fluoride, py h) benzene, **4**, (46 %)

Scheme 2

the benzyl ester. The alcohol was transformed into the triflate and hydroxy lactam **3** was incorporated to provide the substituted β-lactam **6**. The ester was deprotected with Pearlman's catalyst and the corresponding acid was converted to the acid fluoride. Treatment with silyl derivative **4** yielded hydrazide 7.[14]

The removal of the N-silyl group on hydrazide **7a** with TBAF followed by Boc cleavage with TFA led to the seven-membered ring heterocycle[12] (Scheme 3). The former β-lactam nitrogen was acetylated and the benzyl ester was converted to the monomethyl amide to provide the desired γ-turn mimetic **1a**.



a)TBAF, THF b) TFA/anisole (9:1) c) Ac_2O, py (36-51 %) d) H_2, $Pd(OH)_2/C$ e) EDCI, HOBt, $MeNH_2$•HCl (70 %)

Scheme 3

The synthesis of **1b** proved to be more challenging. Hydrazide **7b** did not yield the desired seven-membered ring heterocycle upon exposure to similar reaction conditions as **7a**. The product isolated was acetyl hydrazine **8** (Figure 2) and unreacted starting materials. Confirmation of the uncyclized product was procured from NMR experiments.[15] Cyclization was executed by inverting two of the steps that were employed in the previous γ-turn synthesis.

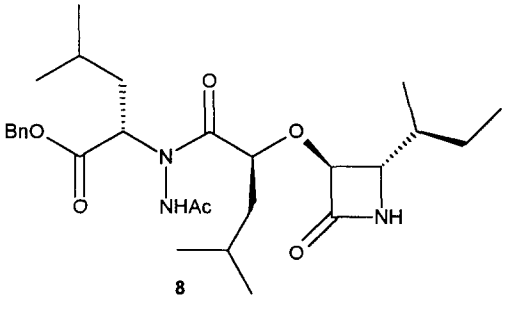


Figure 2

The structures and conformations of the γ-turn mimetics were analyzed by extensive 2D NMR experiments.[16] All protons were assigned specifically using DQF-COSY, TOCSY, and ROESY. Additionally, temperature coefficients ($\Delta\delta/\Delta T$)[17] were measured for the exchangeable amide protons: isoleucine NH and

hydrazide NH. The temperature coefficients for mimetic **1a** are -2.4 ppb and -1.4 ppb respectively. The values for **1b** are -2.2 ppb and -1.1 ppb respectively. The small temperature coefficient values indicate that these protons are protected from the solvent and/or hydrogen bonded.

The synthesis of two γ-turn mimetics was performed based on molecular modeling of the malformins. NMR temperature coefficient studies demonstrated that the amide protons were shielded from the solvent and/or hydrogen bonded. A preliminary biological screen showed compound **1b** displayed 10 % inhibition at 10 μM for Neuropeptide Y.

Acknowledgments

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11. The RMSD, 0.17Å, of the γ-turn templates were compared with ideal γ-turns and the global minimum conformation of abbreviated malformin were obtained at 9 atom positions: Cα(i), C(i), N(i + 1), Cα(i +1), C(i +1), O(i + 1), Cβ(i +1), N(i + 2), and Cα(i + 2).
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