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Synthesis of a conformationally restricted dipeptide analogue and its evaluation as a β-turn mimic

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Abstract—Dichloropyrazinone **3** was converted into a conformationally restricted dipeptide analogue **8** by means of a Diels–Alder strategy. The β-turn characteristics of molecule **8** were examined by molecular modeling and NMR spectroscopy.

The β-turn is one of the three major motifs of peptide and protein secondary structure. It plays a key role in many molecular recognition events including interactions between antigens and antibodies, peptide hormones and their receptors, and enzymes and their corresponding substrates.[1] Due to the often poor bioavailability and low stability of natural peptide therapeutics, much effort has been devoted to the synthesis of peptide mimics to overcome the aforementioned problems.

Because of its biological importance, its compact size and hence synthetic accessibility, the β-turn is an interesting motif to mimick.[1] A β-turn mimic usually consists of a conformationally restrained dipeptide. Based on known β-turn mimics such as piperidinone derivative **1** and the bicyclic lactam **2**, respectively described by Kemp[2] and Germanas[3] (Fig. 1), we developed a method for the synthesis of the structurally related compound **8**. The above-mentioned β-turn mimics contain a 3,6-disubstituted 2-piperidinone ring bearing a *cis*-oriented C-3 amine group and C-6 carboxyl group as core structure.

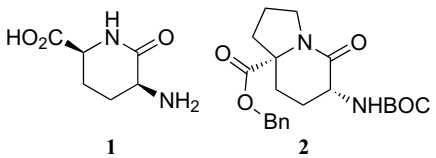

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Figure 1. Dipeptide β-turn mimics.

Our approach to the 3,6-disubstituted 2-piperidinone is based on the pyrazinone chemistry previously developed in our laboratory[4] (Scheme 1). The starting pyrazinone **3** is synthesized from an aminonitril and oxalyl chloride.[4a] The chlorine at the 3-position is substituted by a methyl group by reacting **3** with 1.1 equiv. of Me_4Sn in toluene at 110°C using $Pd(PPh_3)_4$ as a catalyst.[4b] Product **4** was purified by column chromatography (silica gel, gradient hexane/dichloromethane 15/85→dichloromethane→ethyl acetate/dichloromethane 5/95).

The conformational restriction of the system is imposed by a Diels–Alder reaction of ethene on the substituted pyrazinone **4** in toluene (steel bomb, 33 atm, 135°C). The crude imidoyl chloride **5** is converted into **6** by hydrolysis in $CHCl_3$ exposed to air moisture. Evaporation of this reaction mixture and recrystallization (hexane/dichloromethane) yields the pure bicyclic compound **6** (overall yield from **4**: 79%). The secondary lactam moiety in **6** is selectively cleaved upon treatment with an HCl-saturated methanol solution for 12 h. Under these conditions there is no risk of cleaving the benzylated amide function of the piperidinone formed.

In order to prevent recyclization to the bislactam **6** upon neutralization of the reaction mixture, the newly formed primary amine function is trapped as an acetamide by dissolving the evaporated residue from methanolysis in acetic anhydride and adding Et_3N until

Keywords: pyrazinone; Diels–Alder; piperidinone; constrained dipeptide; β-turn mimic.

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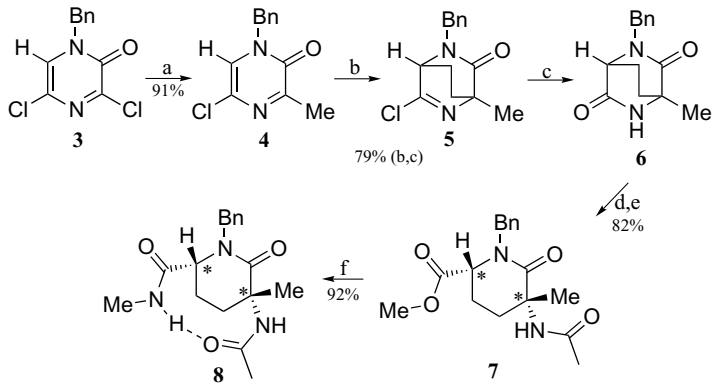

Scheme 1. Me_4Sn, $Pd(PPh_3)_4$, toluene, 110°C; (b) ethene (33 atm), toluene, 135°C; (c) $CHCl_3$, air moisture, rt; (d) HCl/MeOH, rt; (e) Ac_2O/Et_3N, rt; (f) $MeNH_2$ (33%)/EtOH, rt.[5]

no more salts are formed. Removal of the salts by filtration and evaporation of the reaction mixture yields product **7**, which is further purified by crystallization from hexane/dichloromethane. Compound **8** is finally obtained by dissolving **7** in $MeNH_2$ (33%)/ethanol and stirring this mixture for 12 h at room temperature. Upon recrystallization (hexane/dichloromethane) pure **8** is obtained.

The β-turn characteristics of the substituted dipeptide **8** were investigated by molecular modeling and ^{1}H NMR spectroscopy. A β-turn is defined as a tetrapeptide sequence in which the interatomic distance $\alpha C_{(1)}$–$\alpha C_{(4)}$< 7 Å (Fig. 2). A hydrogen bond is often present between $CO_{(1)}$ and $NH_{(4)}$, although open turns lacking this hydrogen bond also exist.[1]

In order to qualify **8** as a β-turn mimic, the structure was checked for the above-mentioned criteria. A modeling study was performed on the simplified molecule **9**[6]

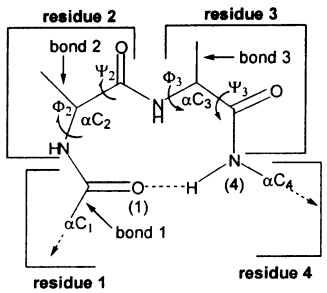

Figure 2.

Figure 3.

(Fig. 3) to estimate the interatomic distance $\alpha C_{(1)}$–$\alpha C_{(4)}$. The starting conformation was preoptimized by putting the amide bonds outside the ring in their more stable *trans* conformation. Energy minimization of the starting geometry resulted in two possible structures in which the main difference was the conformation of the ring system. Based on the axial coupling observed in the ^{1}H NMR spectrum of compound **8** for H-6, conformation A (Fig. 3) was found to be the best approximation of the real geometry of **8**. In this model, the interatomic distance between $\alpha C_{(1)}$–$\alpha C_{(4)}$ is 5.72 Å. This lies well beneath the 7 Å upper limit mentioned in the literature. Also, an intramolecular hydrogen bond between $CO_{(1)}$ and $NH_{(4)}$ can be inferred by the interatomic distance of 2.13 Å.

The presence of a hydrogen bond was further checked by ^{1}H NMR spectroscopy on compound **8**. According to the literature the temperature dependence of the chemical shift of a hydrogen bonded amide proton is small (0 to −4 ppb/°C) compared to the temperature dependence of a solvent exposed proton (<−7 ppb/°C).[7] The chemical shifts of the N*H*CO (singlet at $\delta = 8.47$ ppm) and N*H*Me (quartet at $\delta = 8.46$ ppm) in DMSO-d_6 were recorded at different temperatures. Linear regression on the collected datapoints provided us with the following results: the chemical shift dependence of −3.9 ppb for the N*H*Me is consistent with the presence of a hydrogen bond; the N*H*CO on the other hand is solvent exposed (shift dependence −7.2 ppb/°C).

A final NMR criterion used was the solvent dependency of the chemical shift of the amide protons upon changing the solvent from DMSO to $CDCl_3$. The results are summarized in Table 1. The small shift of the N*H*Me also confirms the presence of a hydrogen bond.

In summary, we were able to synthesize a conformationally restricted Ala-Gly analogue **8** in multigram quantities. Both ^{1}H NMR and modeling studies affirm the proposed β-turn characteristics of this structure. Further research will be directed towards the incorporation of this β-turn mimic in a peptide library.

Table 1. Chemical shift dependence of amide protons of **8**

δ_{NHMe} DMSO (ppm)	δ_{NHMe} $CDCl_3$ (ppm) δ	$\Delta\delta_{NHMe}$ (ppm)	δ_{NHCO} DMSO (ppm)	δ_{NHCO} $CDCl_3$ (ppm)	$\Delta\delta_{NHCO}$ (ppm)
8.46	8.32	0.14	8.47	5.88	2.59

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5. (a) Relative configuration is indicated; (b) all new compounds exhibit satisfactory spectral and analytical data.
6. Calculations were performed with Hyperchem (BIO+-force field). Energy minimizations of **8** did not converge, so we used **9** as a model for **8**. ^{1}H NMR data of **8** are consistent with the conformation A of the piperidinone ring in **9**.
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