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A new spirocyclic proline-based lactam as efficient type II′ β-turn inducing peptidomimetic

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ABSTRACT

A new proline-based spirotricyclic lactam is reported as an efficient type II′ β-turn inducing peptidomimetic. After investigations of the reverse turn properties by computational techniques, the scaffold has been synthesized by a straightforward sequence relying on a key RCM reaction for the construction of the spirocyclic lactams ring. For its conformational properties, the scaffold can be considered a privileged structure to be employed as a mimic of the β-turn motif of the potent antibiotic Gramicidin S.

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1. Introduction

The use of conformationally constrained scaffolds for the preparation of reverse turn mimics is a main topic in medicinal chemistry.[1] Of particular interest is the design of structures, which are able to mimic the β-turn motif,[2] due to the importance of this fragment in proteins folding[3] and in the protein-receptor interaction process. Subclasses of β-turns are further distinguished on the basis of the backbone dihedral angles (φ, ψ) associated with central $i+1$ and $i+2$ positions. Among proposed scaffolds, proline-based spirocyclic lactams, occupying the $i+1$ position of the β-turn, have proven to be very effective in constraining the dihedral angle φ ($i+1$) and ψ ($i+1$) in type II–II′ β-turn inducing systems.[4]

An example of the critical role of the β-turn for the biological activity is showed in Gramicidin S.[5] Gramicidin S (GS) is a cyclodecapeptide antibiotic, which targets the membrane lipid bilayer thanks to its amphiphilic nature.[6] The amphiphilicity of GS is a consequence of an antiparallel β-sheet conformation stabilized by the presence of a type II′ β-turn having a L-proline residue at the $i+2$ position.[7]

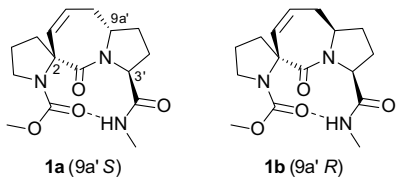

Figure 1. Design of new spirotricyclic β-turn mimics **1a**,**b**.

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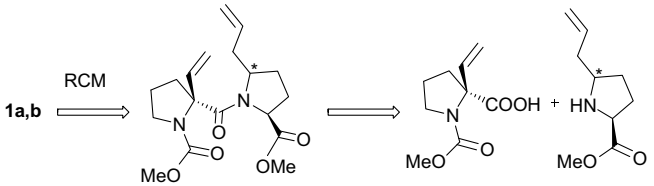


Scheme 1. Retrosynthetic approach to **1a**,**b**.

In our ongoing efforts toward the preparation of β-turn mimics,[8] we describe here the design and the synthesis of a new spirocyclic proline-based lactam that is able to induce a type II′ β-turn conformation. The proposed scaffold is the result of constraining a D-Pro-L-Pro dimer through a 6,7-dihydro-azepin-2-one lactam, thus obtaining a β-turn mimetic with a L-proline residue in the $i+2$ position (**1a,b**, Fig. 1). The use of D-proline in the $i+1$ position is crucial to obtain a type II′ β-turn conformation. Being fixed the C2 and C3′ stereocenters, the influence of the configuration at C9a′ on the reverse turn properties was evaluated.

The structures **1a,b** have been deeply studied by computational techniques to investigate the reverse turn properties. The synthesis of dipeptide mimics **1a,b** has been planned as the result of a straightforward sequence relying on a key RCM reaction for the construction of the spirocyclic lactams ring (Scheme 1).

2. Results and discussion

2.1. Computational studies

The reverse turn mimicry ability of the two diastereoisomers **1a,b** was evaluated by computing[9] the main geometric features of β-turns. The interatomic distance $d\alpha$, here represented by the

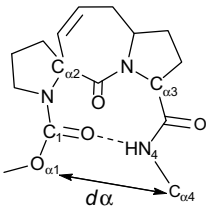


Figure 2. Parameters for the characterization of β-turn propensity of **1a**,**b**.

Figure 3. Low-energy conformers for **1a**,**b**. Hydrogen atoms are omitted.

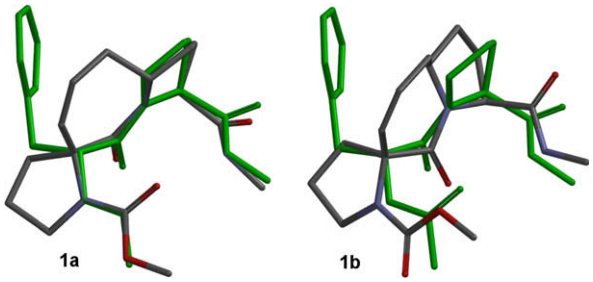


Figure 4. Superimposition of low-energy conformers of **1a**,**b** with X-ray structure of the D-Phe-Pro portion of Gramicidin S (in green). Hydrogen atoms are omitted.

($O\alpha_1$–$C\alpha_4$) distance, should be less than 7 Å and the virtual torsion angle β, defined by C_1–$C\alpha_2$–$C\alpha_3$–N_4, should be $|\beta| < 30°$ (Fig. 2).

Another important feature in stabilizing the reverse turn conformation is the presence of the characteristic hydrogen bond $C_1O\cdots HN_4$ that was estimated by means of the 'hydrogen bonds' function implemented in the software.[10] The computational procedure consisted in an unconstrained Monte Carlo/Energy Minimization conformational search using the molecular mechanics MMFF94 force field[11] in vacuo. For each diastereoisomer, only conformations within 6 kcal/mol of the global minimum were kept. Results are reported in Table 1 as percentage of conformers, which meet the requirements for a generic β-turn.

Results from conformational analysis show the good ability of both **1a** and **1b** to induce a β-turn conformation, with a 100% of conformers having a $d\alpha < 7$ Å. Nevertheless, the lack of conformers able to arrange the intramolecular H bond in compound **1b** is an unfavorable condition in stabilizing the reverse turn structure.

Since we were interested in mimicking the specific type II′ β-turn, we measured the dihedral angle of the amide backbone of the global minima of **1a,b**, comparing them to the values of standard type II′ β-turn. As can be seen from Table 2, the best fit with a standard type II′ β-turn is found for **1a**, while for compound **1b** a large deviation from the standard for the $\psi_{(i+1)}$ and $\psi_{(i+2)}$ angles is revealed. Results of a similarity analysis (Table 3), performed by superimposing the atoms of the amide backbone with standard type β-turns,[12] confirm the excellent ability of **1a** to mimic a type II′ β-turn (score 0.99), while for **1b** it is impossible to assign an univocal β-turn type.

In Figure 3 are reported the structures of the low energy conformers for **1a,b** as obtained from the MM/EM procedure. While for **1a** is clearly visible the desired type II′ β-turn arrangement, in the case of **1b** is evident the cis conformation adopted by the carbamoyl bond of the D-proline residue, which hampers the β-turn disposition.

The efficiency of **1a,b** as analogs of the β-turn motif of Gramicidin S has been evaluated by superimposing their low-energy conformers with the D-Phe-Pro portion of Gramicidin S as obtained by X-ray structure[13] (Fig. 4).

This comparison shows an excellent similarity between **1a** and the β-turn portion of Gramicidin S, especially for the L-proline moiety, while for **1b** a non-optimal superimposition of the amide skeleton is apparent.

All these data suggest that the 9a′*S* configuration present in **1a** should be critical to induce a type II′ β-turn conformation. As a consequence of these studies, we decided to address our interest to the preparation of **1a**.

Table 1
MC/EM conformational analysis for structures **1a**,**b**

Compd	No. of conf. <6 kcal/mol	% $d\alpha < 7$ Å[a]	% $\|\beta\| < 30$[a]	% H-bond[a]
1a	4	100 (4)	50 (2)	25 (1)
1b	4	100 (4)	100 (4)	0 (0)

[a] Results are reported as percentage of conformers, which meet the indicated requirement. The occurrence numbers are given in parentheses.

Table 2
Characteristics of low-energy conformers calculated for **1a**,**b**

	$d\alpha$[a]	β[b]	$\varphi_{(i+1)}$[b]	$\psi_{(i+1)}$[b]	$\varphi_{(i+2)}$[b]	$\psi_{(i+2)}$[b]
II′ β-turn	4.75	1.05	60	−120	−80	0
1a	4.87	1.07	57.57	−111.85	−81.13	−17.08
1b	4.55	−13.88	61.56	−171.97	−83.64	70.76

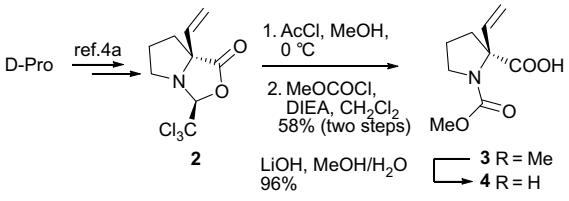
[a] The distances are measured in Å.
[b] Torsion angles are reported in °.

Table 3
Scores obtained from similarity analysis of **1a**,**b** with standard type β-turns

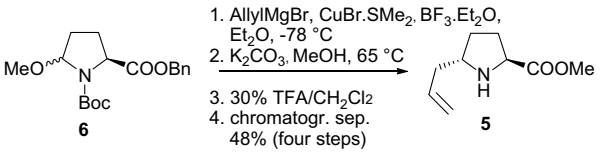
	β-turn type							
	I	II′	II	II′	III	III′	V	V′
1a	0.89	0.81	0.75	0.99	0.91	0.76	0.68	0.76
1b	0.73	0.74	0.77	0.78	0.72	0.74	0.63	0.77

2.2. Synthesis

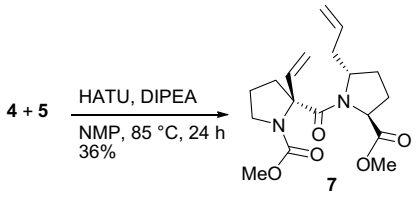
The spiro 2-vinyl D-proline derivative **2**[14] was prepared starting from D-proline according to a reported procedure.[4a] This intermediate was treated with AcCl in MeOH and subsequently with methyl chloroformate to cleanly afford the methyl carbamate **3**. The hydrolysis of the methyl ester function of **3** allowed to obtain the desired acid **4**[15] in satisfactory yields (Scheme 2).



Scheme 2. Synthesis of acid **4**.



Scheme 3. Synthesis of amine **5**.



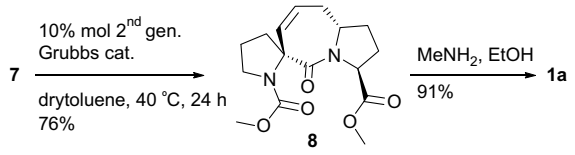
Scheme 4. Synthesis of amide **7**.

The 5 allyl-proline derivative **5** was obtained through a stereoselective allylation on precursor **6**[16] using allylmagnesium bromide with $CuBr{\cdot}Me_2S$ complex in the presence of BF_3 etherate at −78 °C, according to slight modifications of a reported protocol.[17] The reaction afforded an inseparable cis/trans mixture of the desired 5-allyl-*N*-Boc-proline ester. After transesterification in refluxing methanol with an excess of K_2CO_3, the Boc was removed with 30% TFA in CH_2Cl_2. Chromatographic separation afforded the desired major trans diastereoisomer **5**[18] (24:1 dr) in satisfactory overall yield (Scheme 3).

The coupling between the amine **5** and the acid **4** (Scheme 4) proved to be a non-trivial issue. We tested different classical peptide condensating agents, but the desired product **7**[19] could be achieved in acceptable yield (36%) only with the use of HATU and DIPEA in NMP. While the PCl_5/pyridine system led to the formation of an inseparable mixture of products, the use of BOP-Cl[20] led to no reaction. By employing DCC/HOBT or BOP, we could only recover the HOBT ester derivative of the acid **4**. This fact points out the steric hindrance of **5** as the main reason of the difficulty of this reaction. Product **7** could also be obtained with the use of PyBOP (13%) and HBTU (9%). Notably, we could observe that the stereochemical assessment of **5** is critical for the outcome of this reaction: in fact, the coupling of **4** with the (3*S*,5*S*) cis diastereoisomer of **5** afforded the amide product in 64% yield.

Product **7** was then submitted to RCM cyclization with 2nd generation Grubbs catalyst in toluene at 40 °C to afford **8** in good yields. By reaction of **8** with methyl amine in ethanol, the target compound **1a**[21] was ultimately achieved (Scheme 5).

The ^1H NMR spectrum of **1a** showed a low field (7.72 ppm) signal for the amide proton, thus suggesting the involvement of this hydrogen in an intramolecular H-bond, as predicted by computational studies for the type II′ β-turn conformation. To further investigate the presence of this H-bond, the temperature dependence[22] of the chemical shift of the amide proton of **1a** was examined by recording four ^1H NMR spectra between 303 K and 333 K. All analyses were performed in a 3.0 mM solution in $CDCl_3$. The low value of the resulting variable temperature coefficient ($\Delta\delta/\Delta T = -3.8$ ppb/K) can be assumed as an evidence of the presence of an intramolecular H-bond.



Scheme 5. Synthesis of target compound **1a**.

3. Conclusions

In this work, we described the design and the synthesis of the new spirocyclic proline-based lactam **1a**. Computational studies clearly indicated that this scaffold can efficiently adopt a type II′ β-turn conformation. Compound **1a** was synthesized in few steps starting from suitable proline derivatives through a RCM reaction. This type II′ β-turn inducing peptidomimetic, having a L-proline residue in the $i+2$ position, is a good candidate in replacing the β-turn motif of the potent antibiotic Gramicidin S. Further studies on the preparation and conformational analysis of Gramicidin S derivatives containing the scaffold **1a** will be reported elsewhere.

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10. Hydrogen bonds are defined as non-bonded contacts between a nitrogen or oxygen and an hydrogen attached to nitrogen or oxygen, separated by a distance ranging from 1.6 Å to 2.1 Å and making an X–H···Y (X,Y = N,O) angle >120°.
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14. *Compound* **2**: NMR data were in agreement with previously reported data for ent-**2** (see Ref. 4a). $[\alpha]_D^{20}$ −45.9 (*c* 0.25, $CHCl_3$).
15. *Spectral data for* **4**: ^1H NMR (400 MHz, $CDCl_3$, major rotamer) δ 10.01 (br s, 1H), 5.82 (m, 1H), 5.22–4.98 (m, 2H), 3.77 (s, 3H), 3.61–3.54 (m, 2H), 2.35–2.20 (m, 1H), 2.11–1.92 (m, 2H), 1.72–1.55 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) δ 171.8, 155.1, 135.3, 113.8, 79.0, 52.4, 47.4, 38.8, 23.1. HRMS Calcd for $C_9H_{13}NO_4$: 199.0845, found: 199.0852.
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19. *Spectral data for* **7**: ^1H NMR (400 MHz, $CDCl_3$, major rotamer) δ 5.92–5.64 (m, 2H), 5.46–5.03 (m, 4H), 3.82–3.54 (m, 10H), 2.41–2.13 (m, 4H), 2.11–2.01 (m, 1H), 1.99–1.84 (m, 2H), 1.75–1.53 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, major rotamer) δ 175.8, 172.0, 154.9, 139.8, 137.2, 119.6, 113.4, 78.8, 60.1, 60.0, 53.1, 52.3, 52.0, 37.8, 30.1, 29.1, 26.0, 23.7. HRMS Calcd for $C_{18}H_{26}N_2O_5$: 350.1842, found: 350.1836.
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21. *Spectral data for* **1a**: ^1H NMR (400 MHz, $CDCl_3$) δ 7.72 (br q, J = 8.9 Hz, 1H), 5.90–5.78 (m, 1H), 5.12 (br d, J = 11.7 Hz, 1H), 4.58 (br t, J = 12.6 Hz, 1H), 4.22 (dd, J = 11.6, 6.8 Hz, 1H), 3.78–3.60 (m, 5H), 2.52–2.35 (m, 2H), 2.29 (d, J = 8.9 Hz, 3H), 2.40–1.35 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 163.3, 154.8, 130.7, 127.1, 77.1, 60.5, 54.1, 36.5, 32.4, 31.3, 29.1–24.0 (5C). HRMS Calcd for $C_{16}H_{23}N_3O_4$: 321.1689, found: 321.1682.
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