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Design, Synthesis, and Conformational Analysis of 3-*Cyclo*-Butylcarbamoyl Hydantoins as Novel Hydrogen Bond Driven Universal Peptidomimetics

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A collection of systematically substituted 3-*cyclo*-butylcarbamoyl hydantoins was synthetized by a regioselective multicomponent domino process followed by easily coupling reactions. Calculations, NMR studies and X-ray analysis show that these scaffolds are able to project their side chains similar to common secondary structures, such as the α-helix and β-turn, with favourable enthalpic and entropic profiles.

In modern medicinal chemistry, a well-established strategy to overcome the drawbacks associated with the use of peptides as drugs is the synthesis of peptidomimetics that efficiently mimic protein secondary structures.[1] Recently, this strategy became increasingly important, even if challenging, in the design of protein-protein interaction (PPI) inhibitors, since PPIs are involved in most biological processes. Despite the large and shallow surface area of contact, the PPI free Gibbs energy binding depends mainly on the interaction of certain protein side chains present in secondary structural elements, *i.e* hot spots.[2] In particular, structural mimetics, where small-molecule scaffolds replace the entire peptide backbone encompassing minimalist and universal mimetics, have received great attention.[3] Since early examples of structural β-turns[4] and α-helix[5] mimetics, several minimalist mimetics mimicking all type of secondary structures, as well as some universal mimetics, have been developed in the last decade.[6] In particular, the development of new universal peptidomimetics is highly intriguing because, being able to mimic different protein secondary structures and to adapt their conformations through rotation around a few degrees of freedom, they facilitate high throughput screening (HTS) against different targets, overall when the target binding conformation is not well characterized.[3]

Inspired by minimalist hydrogen bond driven β-turn mimetic *trans*-pyrrolidine-3,4-dicarboxamide developed by Boger et al.,[7] we hypothesized that the introduction of two carbonyl groups in the five-membered ring could 1) generate alternative hydrogen bond networks facilitating the mimicry of different secondary structures, and 2) improve the "druggability" of the mimics. Accordingly, we designed 3-*cyclo*-butylcarbamoyl hydantoin scaffold **1** (Figure 1a) as structural privileged universal mimetic scaffold that would possess the following properties: 1) presentation of the side chains mimicking secondary protein structures, such as the α-helix and β-turn; 2) sufficient flexibility for adapting to a broad range of kinetically and thermodynamically accessible conformations, but also 3) limited degrees of freedom (favourable entropic profile); 4) facile synthesis and easily incorporation of a wide range of amino acid side chains, suitable for a combinatorial approach (Figure 1b,c).[8] Taken together, all these features will possibly facilitate the synthesis of libraries of compounds **1** for HTS against different targets.[6]

3-*Cyclo*-butylcarbamoyl hydantoins **7**, precursors of final mimetics **1**, were synthesized exploiting a regioselective sequential multicomponent (MC) domino process recently developed by us (Table 1. For the detailed mechanism of the MC process, see Scheme S1).[9] Reaction between α-azido-*cyclo*-butyl carboxylic esters **2** and isocyanates **3** in the presence of Ph_3P affords carbodiimides **4**, which react *in situ* with fumaric acid monoesters producing the 3-*cyclo*-butylcarbamoyl hydantoin scaffold. In this work, we introduce the fumaric acid mono-*p*-nitrophenyl ester **5**. The activated ester readily reacts *in situ* when an equimolar amount of an amine is added to the reaction medium, once intermediate **6** is formed (see SI).

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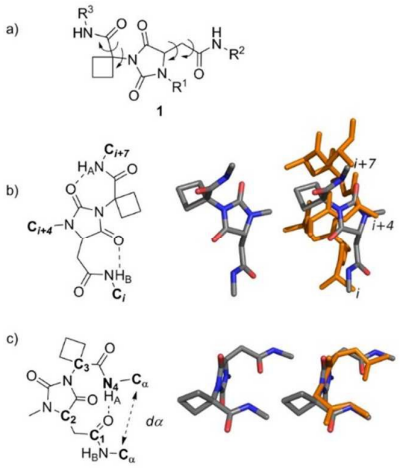
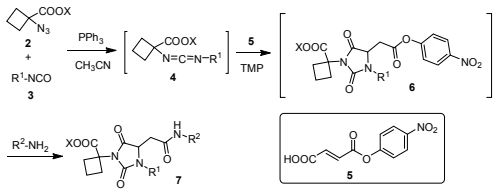


Figure 1. a) General structure and flexibility of 3-*cyclo*-butylcarbamoyl hydantoin scaffold **1**; b) simulated low energy α-helix conformer (R^1 = R^2 = R^3 = Me, grey) and its superimposition with an ideal α-helix (orange); c) simulated low energy β-turn conformer (R^1 = R^2 = R^3 = Me, grey) and its superimposition with an ideal β-turn (orange).

Table 1. MC domino synthesis of intermediates **7.**



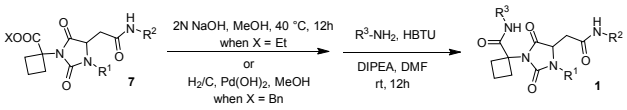
Entry	R^1	R^2	X	Product	Yield (%)[a]
1	Et	*p*-MeO-benzyl	Et		73
2	Et	phenethyl	Bn		77
3	Bn	phenethyl	Bn		75
4	Bn	tryptamine	Bn		69
5	Bn	c-hexyl	Bn		81
6	1-naphthyl	n-hexyl	Bn		77
7	$-CH_2CO_2Et$	phenethyl	Bn		63

[a]Isolated yields.

In this way, we were able to obtain 3-*cyclo*-butylcarbamoyl hydantoins **7a-g** in good yields, through a sequential four-component process.

The ester function of compounds **7a-g** can be easily hydrolysed by base (X = Et) or through hydrogenolysis (X = Bn), and the resulting carboxylic acids coupled with different amines affording final mimetics **1** (Table 2).[10]

Table 2. Synthesis of mimetics **1**.



Entry	Ester	R^3	Product	Yield (%)[a]
1	**7a**	benzyl		81
2	**7a**	*c*-hexyl		74
3	**7a**	butyl		89
4	**7a**	HO-Gly-		65[b]
5	**7b**	hexyl		76
6	**7c**	tryptamine		69
7	**7d**	tryptamine		65[c]
8	**7d**	tyramine		64
9	**7e**	phenethyl		78
10	**7f**	triptamine		81
11	**7g**	tyramine		74

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[a]Isolated yields. [b]*Via* coupling with BnO-Gly-H followed by hydrogenolysis; [c]*Via* coupling with *N*-Boc-1,6-hexamethylendiammine followed by Boc removal with TFA.

The synthetic procedure presented possess the suitable requirements to be used for the preparation of a wide library of structural mimetics. All the natural (and unnatural) amino acid side chains are available based on a MC domino process where the side chains come from easily accessible amines (R^2, R^3) and isocyanates (R^1). Moreover, compounds **1a-k** follow Lipinsky's rule of five (Table S1).[11] In particular, the octanol-water partition coefficients (log P) of all compounds are smaller than 5, rendering them promising candidates in drug discovery.

In order to evaluate the ability of the scaffolds to mimic classical peptide secondary structures, we performed a preliminary computational study of model compound **A** (R^1 = R^2 = R^3 = Me) and compounds **1a-k** using Molecular Mechanics Merck Force Field (MMFF) for energy minimization. We mainly focused on the ability to mimic α-helix and β-turn secondary structures (a more exhaustive analysis of all the possible secondary structures mimicked, according to Burgess at al., is reported in Table S2).[3] The three-diversification points of the scaffold C*i*, C*i+4* and C*i+7* were associated to the *i*, *i*+4 and *i*+7 residues of an ideal α-helix (Figure 1b). The ideal interatomic distances *i*-*i*+4 = 6.2 Å, *i*-*i*+7 = 10.3 Å and *i*+4-*i*+7 = 5.8 Å were used as parameters. For the β-turn conformation, the interatomic distance *d*α < 7Å was used as a limit (Figure 1c). The absolute value of the dihedral angle C_1-C_2-C_3-N_4 β < 30° was also evaluated as a more stringent condition. All the conformers within 10 kcal/mol from the global minimum from the conformational analysis were kept. Results of the geometrical measurements are reported in Table 3 as percentage of the structures that meet above requirements.

Table 3. Percentage of conformers that meet specific parameters typical of α-helix/β-turn conformations.

mimic	% *i*-*i*+4[a]	% *i*-*i*+7[a]	% *i*+4-*i*+7[a]	% α-helix[b]	% *dα* <7Å	angle *β* <30°
A	87	26	46	17	25	17
1a	31	13	36	10	40	15
1b	78	24	63	19	36	15
1c	86	14	55	11	41	19
1d	88	20	65	17	39	11
1e	56	27	61	12	14	14
1f	88	19	53	19	35	21
1g	83	19	66	18	17	16
1h	84	37	39	28	13	24
1i	90	25	38	20	19	16
1j	96	11	75	11	4	6
1k	71	14	48	9	19	10

[a]Values in the ± 10% range with respect to the ideal distance were considered; *i*-*i*+4 = 5.6-6.8 Å; *i*-*i*+7 = 9.3-11.3 Å; *i*+4-*i*+7 = 5.2-6.4 Å. [b]Percentage of conformers having the three interatomic distances in the correct range.

For model compound **A**, 17% of conformers can adopt a α-helix conformation, whereas another 25% of structures are able to mimic a β-turn (a total of 42% of the conformers are potential peptidomimetics). Similar results were obtained for all compounds, thus confirming the ability of the scaffold to induce a preferential conformation regardless the nature of the residues. The highest success was recorded for the *i*-*i*+4 distance. For compounds **1b**, **1i**, **1k** and model compound **A**, a more accurate study was performed and the results summarized in Table 4. The first five low energy conformers (for both α-helix and β-turn structures) were submitted to HF/3-21G* full optimization followed by DFT/B3LYP-6-31G* single point energy calculation. The α-helix motif is the most stable for all compounds, but the β-turn conformations are easily accessible at room temperature being only 0.048 to 0.246 kcal/mol higher in energy. This feature is essential in designing minimalist/universal peptidomimetics.

Table 4. Comparison of α-helix/β-turn calculated free energies.

mimic	α-helix mimic energy (kcal/mol)	*β*-turn mimic energy (kcal/mol)
A	0.00	0.954
1b	0.00	0.067
1i	0.00	0.048
1k	0.00	0.246

For the secondary structure stabilization, different intramolecular hydrogen bonds can be established and two different patterns have been identified: 1) two H-bonds involving both carbonyl oxygens of the hydantoin ring stabilizing the α-helix, and 2) another H-bond between C_1=O···H_AN_4, not involving carbonyl of the hydantoin ring, which stabilizes the β-turn conformation (Figure 1b,c). To assess the presence of these intramolecular hydrogen bonds in solution, we performed variable temperature (VT) ^{1}H-NMR analysis on compounds **1b** and **1k**. Typically, solvent accessible protons exhibit a Δδ/ΔT > 5 ppb/K, whereas Δδ/ΔT < 5 ppb/K is found for protons involved in H-bonds. The values found in **1b** and **1k** for NH_A (4.0 and 4.4) were smaller than those found for NH_B (4.5 and 5.1), thus indicating a preference for NH_A to be involved in hydrogen bond formation (Figures S1 and S2). This observation is in agreement with an equilibrium between the open (α-helix, both NH_A and NH_B are hydrogen bonded) and folded (β-turn, only NH_A H-bond is present) conformations in solution. The 2D NOESY spectrum of compound **1k** was also recorded. No significant intramolecular long range contact was detected, thus supporting the conclusion that no preferential conformation is adopted.[12]

For compounds **1i** and **1k**, we were able to obtain suitable crystals for X-ray analysis from 1:1 water/methanol solutions as colourless prisms (Figure 2a,b, respectively). Notably, in the solid state, both structures adopted a β-turn conformation with the presence of the characteristic intramolecular C_1=O···H_AN_4 H-bond, with N-H···O distances in the range of 2.0 and 2.3 Å (Table S3). The intramolecular hydrogen bond persisted during the crystal formation even under the influence of relatively large intermolecular packing forces and

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the presence of a highly competitive protic solvent (MeOH) in the crystallization solution. The solid state parameters, such as interatomic distance $d\alpha$, virtual torsion angle β, and dihedral angles ϕ and φ of compounds **1i**,**k** were compared to ideal β-turn conformations (Table 5).

Table 5. Comparison of the main geometrical features between an ideal type II β-turn and crystal structures of **1i**,**k**.

Compound	$d\alpha$ (Å)	$\lvert\beta\rvert$(°)	ϕ_{i+1}	φ_{i+1}	ϕ_{i+2}	φ_{i+2}
Ideal value	< 7	< 30	-60	120	80	0
1i	5.259(1)	1(1)	-53(1)	125(1)	77(1)	19(1)
1k	5.265(1)	4(1)	-47(1)	122(1)	86(1)	20(1)

The values of the distances $d\alpha$ and of the virtual torsion angle β fully comply with the β-turn arrangement. A detailed analysis of the φ and ϕ dihedral angles defines the presence of the β-turn conformation of type-II'. The values of φ_{i+1}, ϕ_{i+1} and φ_{i+2}, ϕ_{i+2} show a higher distortion with respect to the ideal values for the second couple, that may be related to the geometrical constraint of the five-membered ring, which is almost planar.

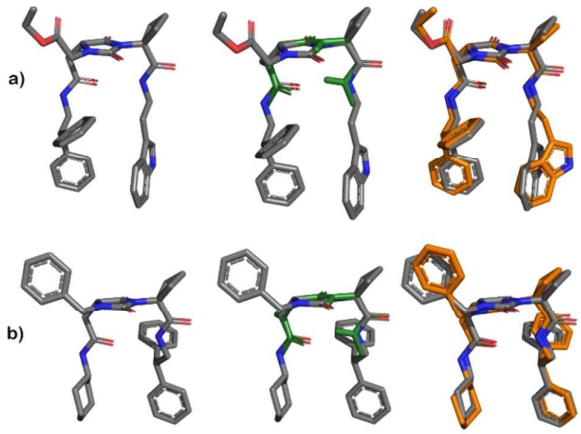

Figure 2. Crystal structure (grey) of **1k** (a) and **1i** (b), and their superimposition with an ideal type-II' β-turn (green) and optimized calculated structure (orange).

Finally, X-ray crystal structures of **1i** and **1k** were superimposed with an ideal type-II' β-turn and β-turn conformer of the same compound as obtained by DFT calculations (Figure 2a,b, respectively). We found a significant geometrical correspondence between the three-dimensional arrangements of the structures, as provided by the RMSD (root-mean-square deviation) values of 0.26Å/0.18Å (with type II' β-turn) and 0.68Å/0.65Å (with DFT structures), respectively, for the superimposition of the heavy atoms. Overall, these results confirm that 3-*cyclo*-butylcarbamoyl hydantoins **1** have a strong tendency to adopt a β-turn conformation, even if accurate modeling studies suggest a slight propensity for a α-helix conformation.

In conclusion, a route for the synthesis of 3-*cyclo*-butylcarbamoyl hydantoin scaffold **1** was devised that involves a regioselective, multicomponent domino process followed by facile couplings. The synthetic pathway is modular, thus suitable for the combinatorial synthesis of these compounds, and a variety of natural and unnatural amino acid side chains can be easily placed in key positions. Molecular modeling showed that these scaffolds could adopt kinetically and thermodynamically accessible conformations mimicking those of the secondary structures with only moderate loss of entropy. In particular, conformations where R^2, R^1, and R^3 substituents overlap with the *i, i*+4, and *i*+7 side-chains in a α-helix are slightly favoured according molecular mechanics, whereas the β-turn conformation is adopted in solid state, as evidenced by X-ray analysis. For the features stated above, 3-*cyclo*-butylcarbamoyl hydantoin scaffold **1** could be considered a new class of effective universal peptidomimetics. Application of mimics **1**, as well as the synthesis, conformation analysis, and application of similar mimetics having other carbocycles in position 3 will be reported in due course.[13]

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Conflicts of interest

There are no conflicts to declare.

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