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Synthesis and Structural Study of a New β-Turn Inducer Peptidomimetic Incorporating 1-Amino-1-aminomethylcyclohexane †

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Abstract: A new β-turn inducer peptidomimetic incorporating 1-amino-1-aminomethylcyclohexane (compound **3**) was synthetized and structurally studied. It was established that there is a rapid conformational equilibrium between the expected β-turn inducer structure and other extended conformations.

Keywords: amino acids; peptides; 1,2-diamines; supramolecular chemistry

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1. Introduction

The search for structures that fold in the form of peptide turns, in which α-amino acids are totally or partially replaced by restrictions made up of β- or γ-amino acids is of great interest. But so are those structures in which α-amino acids are replaced by non-amino acid molecules and which also give rise to peptide turns. The interest, in both cases, is due to the limitations of α-peptides: low structural diversity, due to the limited number of proteinogenic α-amino acids, as well as remarkable conformational flexibility and low metabolic stability.

Some examples of peptidomimetics, including β-turn-inducing structures containing two urea fragments connected by the D-prolyl-*cis*-1,2-diaminocyclohexane dimer (D-Pro-DACH) [1], are listed in Figure 1. This cluster adopts a well-defined β-turn or reverse-turn conformation.

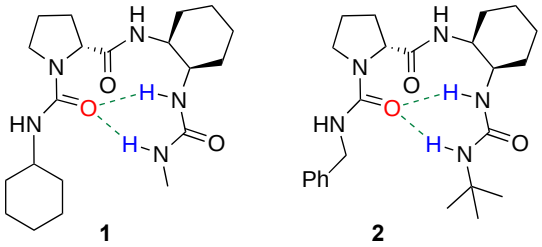

Figure 1. β-turn-inducing structures containing two urea fragments.

It is a turn-inducing cluster consisting of two urea fragments linked by a subunit that combines D-proline with a 1,2-diaminocyclohexane. This allows the two urea units to be

close enough in space to form two hydrogen bonds between them, defining 10- and 12-membered rings, which stabilize the turn.

Our interest in the generation of β-turns with modulable polarity led us to propose the synthesis and structural study of β-turn **3** (Figure 2), analogous to those in Figure 1, but with modulable polarity.

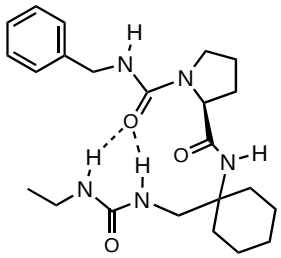

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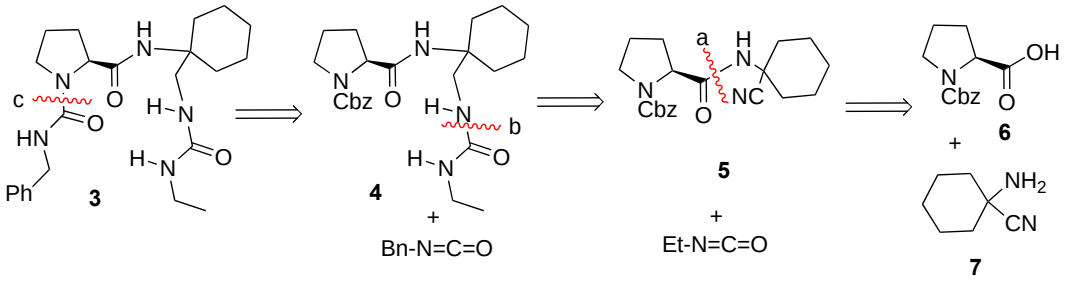
Figure 2. Our β-turn-inducing structures candidate.

The structural similarity of our compound **3** with compounds **1** and **2** (Figure 1) led us to think that it would also adopt a β-turn structure. The additional contribution in our case would be that the β-turn thus generated would foreseeably have the ability to modulate its polarity, depending on the substitution at the *N* atom of the piperidine ring.

The synthesis plan for our diurea **3** is shown in Scheme 1.



Scheme 1. Synthetic plan for target **3.**

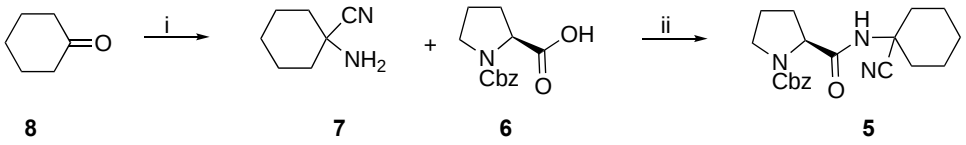
This plan will involves carrying out the following synthetic operations:

1. Coupling of α-aminonitrile **7** with commercial *N*-Cbz-L-proline **6**, to form the strategic bond "*a*" of compound **5**.
2. Reduction of the cyano group to amine and subsequent coupling with ethyl isocyanate to form the strategic bond "**b**" to generate compound **4**.
3. Finally, removal of the Cbz protecting group and coupling with benzyl isocyanate, leading to the formation of the strategic bond "*c*" and thus generating the desired diurea **3.**

2. Results and Discussion

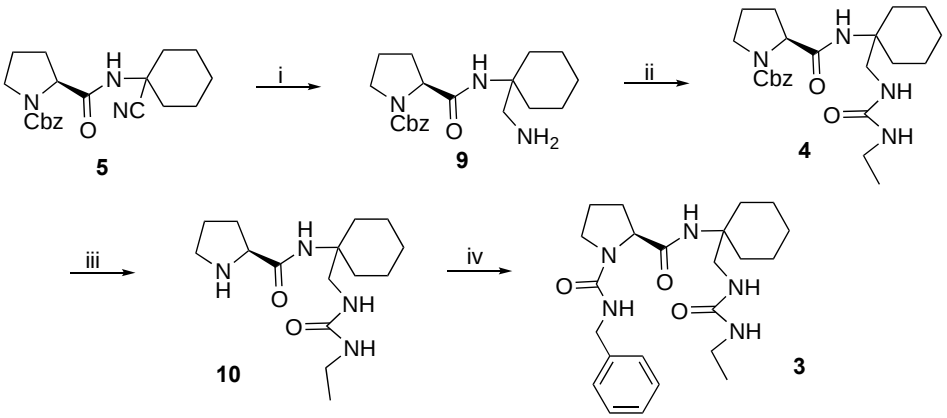
2.1. Synthesis of Compound 3

According to our synthetic approach, as shown in Scheme 2, aminonitrile **7** was obtained from commercial cyclohexanone, following a procedure described in the literature. The coupling of this aminonitrile with commercial *N*-Cbz-L-proline (**6**) was then carried out, using isobutyl chloroformyate as coupling agent and NMO as base, leading to the expected aminonitrile 5, with a yield of 73%.



Scheme 2. Conditions: (i) NH_4Cl, NaCN, dry MeOH, rt., 20 h. (ii) isobutyl chloroformiate, NMO, dry CH_2Cl_2, 0 °C to rt, 5 h (73%, steps).

Next (Scheme 3), aminonitrile **5** was subjected to a reduction reaction of its cyano group to amine, using $NaBH_4$ as reducing agent in the presence of $CoCl_2$. The amine **9**, obtained at 68%, was treated directly with ethyl isocyanate, leading, in 70% yield, to the expected compound **4**.



Scheme 3. Conditions: (i) $NaBH_4$, $CoCl_2$, dry MeOH, rt, 30 min. (68%). (ii) Et-N=C=O, Et_3N, dry CH_2Cl_2, rt, 18 h (70%). (iii) H_2/10% Pd-C, MeOH, rt, 30 min. (iv) Bn-N=C=O, Et_3N, dry CH_2Cl_2, rt, 18 h (48%).

Following our synthetic approach, the Cbz protecting group was removed from **4** by Pd-C catalysed hydrogenation. The amine **10** thus obtained was directly reacted with benzyl isocyanate, yielding the desired peptidomimetic **3** (48%).

2.2. Structural Study of Compound 3

Once we had synthesized, peptidomimetic **3**, we decided to carry out a structural study to check whether it presents a β-turn structure in solution.

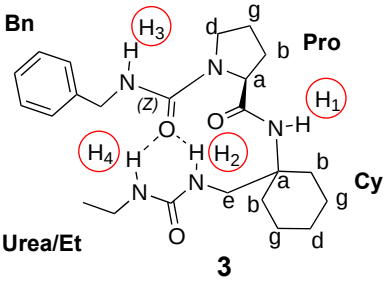


Figure 3. Structure of peptidomimetic **3**, detailing the nomenclature system used for its structural study. (For the units: Urea/Ethyl, Cy, Pro and Bn. For the positions in each unit: Greek alphabet. For NH: H_1, H_2, H_3 and H_4).

The study was started by acquiring NMR experiments (^1H, ^{13}C and TOCSY) leading to the assignment of the signals of the compound (Figure 4 and Table 1).

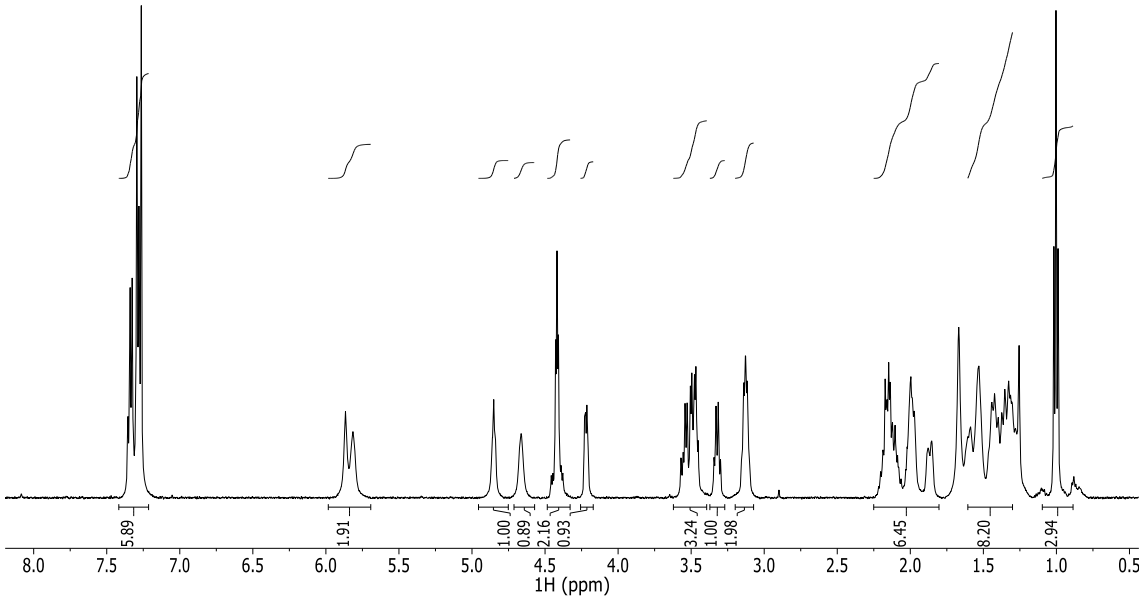


Figure 4. ^{1}H NMR spectrum of compound **3** at +25 °C ($CDCl_3$, 500 MHz).

Table 1. Assignment of the ^{1}H NMR signals of compound **3** (+25 °C, $CDCl_3$, 500 MHz).

Atom	δ^{1}_{H} (ppm)
Bn: Ph	7.22–7.42 (m, 5H)
Bn: CH_2	4.31–4.51 (mAB, 2H)
Bn: H^N	4.85 (t, *J* = 5.7 Hz, 1H, NH)
Pro: α	4.22 (dd, *J* = 7.8, 3.7 Hz, 1H)
Pro: β	1.81–2.23 (m, 2H)
Pro: H	1.81–2.23 (m, 2H)
Pro: δ_1	3.32 (q, *J* = 7.3 Hz, 1H)
Pro: δ_2	3.43–3.63 (m, 1H)
Cy: β	1.29–1.61 (m, 2H)
Cy: β	1.81–2.23 (m, 2H)
Cy: γ	1.81–2.23 (m, 2H)
Cy: γ	1.81–2.23 (m, 2H)
Cy: δ	1.81–2.23 (m, 2H)
Cy: ε	3.43–3.63 (m, 2H)
Cy: α-H^N	5.87 (s, 1H, NH)
Cy: ε-H^N	5.82 (s, 1H, NH)
UREA: Et-CH_2	3.12 (dt, *J* = 11.7, 6.0 Hz, 2H)
UREA: Et-CH_3	1.00 (t, *J* = 7.2 Hz, 3H)
UREA: Et-H^N	4.61–4.74 (m, 1H)

Having carried out the assignment of the ^{1}H NMR signals of compound **3**, we turned our attention to the key experiments, in order to determine the existence of a β-turn structure.

- Serial dilution

In order to find out whether our compound **3** aggregates in solution, which could interfere with studies of its possible intramolecular interactions, ^{1}H NMR experiments were carried out in $CDCl_3$ at +25 °C and at different concentrations (1, 2, 5, 10 and 20 mM). These experiments are shown in Figure 5, and from them it can be concluded that:

1. There is little change with concentration.
2. Between 1 and 5 mM there are hardly any changes, so it can be concluded that at these concentrations there is no aggregation and the molecules are found as monomers in solution.
3. When going from 5 to 10 and 20 mM, some signal shifts, which may mean that above 10 mM aggregation does occur.

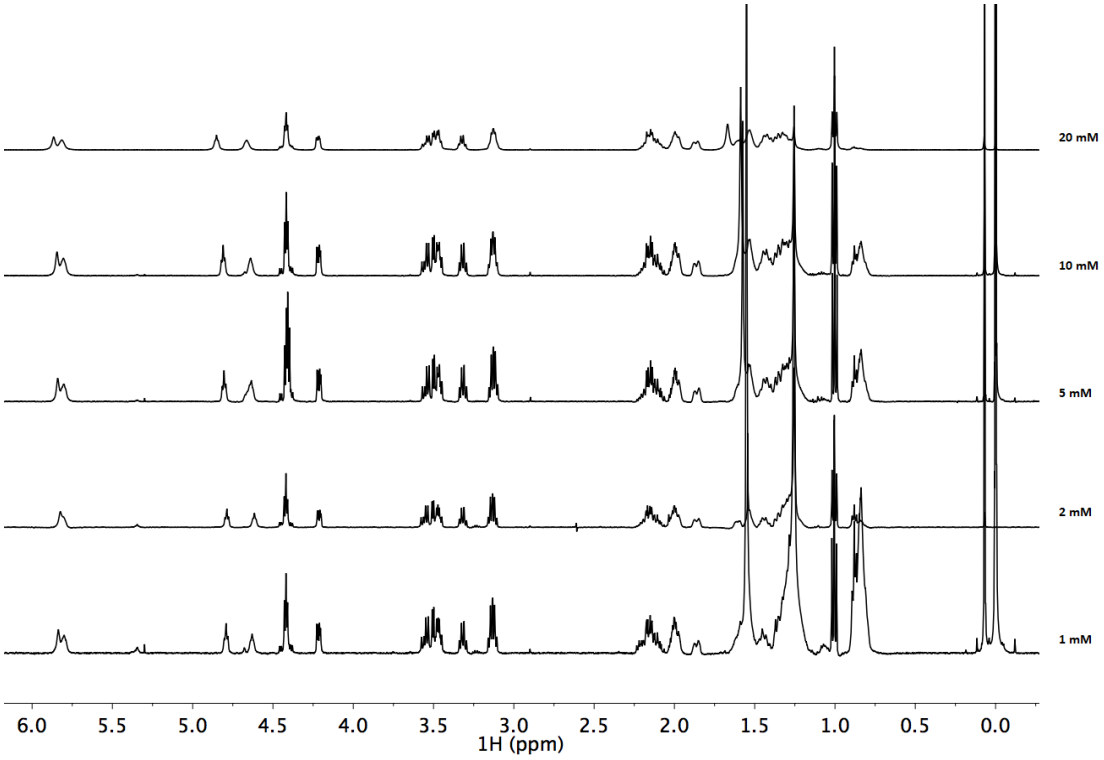


Figure 5. ^{1}H NMR of compound **3** in $CDCl_3$ at +25 °C and at different concentrations (1, 2, 5, 10 and 20 mM).

Therefore, temperature coefficients (T-coef) were determined with 2 mM concentration samples and 2D spectra (TOCSY and NOESY) with 5 mM concentration samples.

- Temperature coefficients (T-coef)

To evaluate the existence of intramolecular hydrogen bonds that can stabilise a β-turn secondary structure for our compound **3**, we now determine the T-coef ($-\Delta\delta/\Delta T$). For this purpose, ^{1}H NMR experiments were performed using water- and acid-free $CDCl_3$, at a concentration of 2 mM and in a temperature range from −10 to 45 °C (with 5 °C increments). The results obtained are shown in Figure 6 and Table 2.

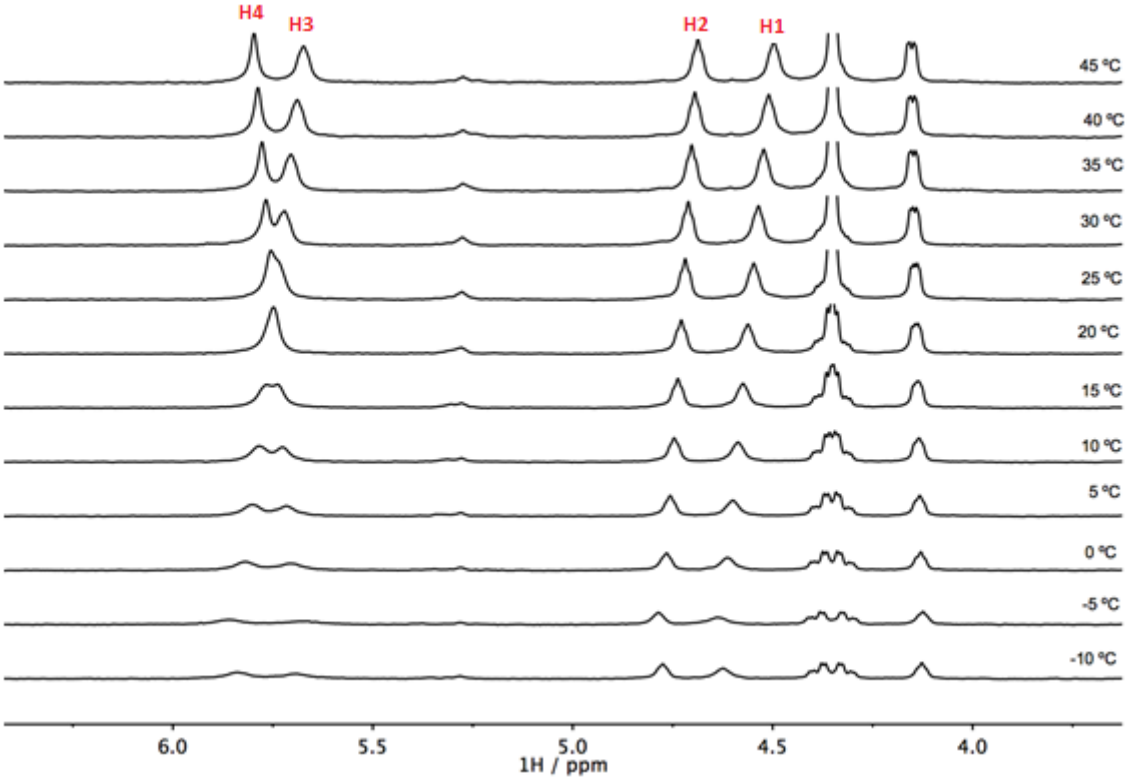


Figure 6. Variable temperature 1H NMR experiment of compound **3**. Temperatures, from bottom to top: −10 to 45 °C (CDCl3, 500 MHz). The resonances of the H^N.

The T-coefs (-Δδ/ΔT) of the four nitrogen-bonded protons (H^N) in our molecule are listed in Table 2 and Figure 7. The values of the temperature coefficients are less than 5 ppb/K for the four protons studied, which indicates that all of them may be either forming hydrogen bonds or not exposed to the solvent.

Table 2. Temperature coefficients of the H^N protons of compound **3.**

Name	δ-max (+45C)	δ-min (−10C)	range T /°C	T-coef (ppb/K)
H_4	4.49	4.63	55	−2.5
H_3	4.69	4.77	55	−1.5
H_2	5.67	5.86	55	−3.5
H_1	5.80	5.69	55	+2.0

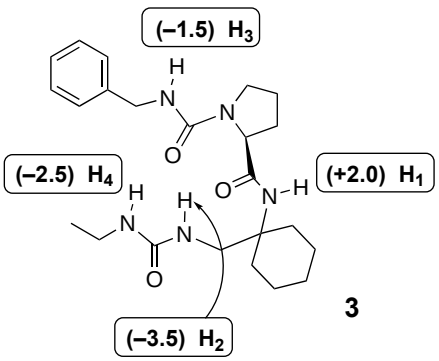


Figure 7. Values of the temperature coefficients of the proton H^N protons of compound **3.**

- Titration with DMSO-d_6

In order to shed light on the possibility that the HNs of our compound are forming intramolecular hydrogen bonds, we decided to perform a titration experiment with DMSO-*d6*. This experiment will allow us to observe the interaction of the HN protons of our compound with DMSO-*d6* and, therefore, how engaged they are in the formation of intramolecular hydrogen bonds [2].

For this, to a 2 mM solution of **3** in $CDCl_3$ (600 µL), amounts of DMSO-*d6* were gradually added, making additions of 5 µL, 10 µL and finally 20 µL. After each addition, the ^1H NMR spectrum was measured to see the change in HN proton shift. (Figure 8).

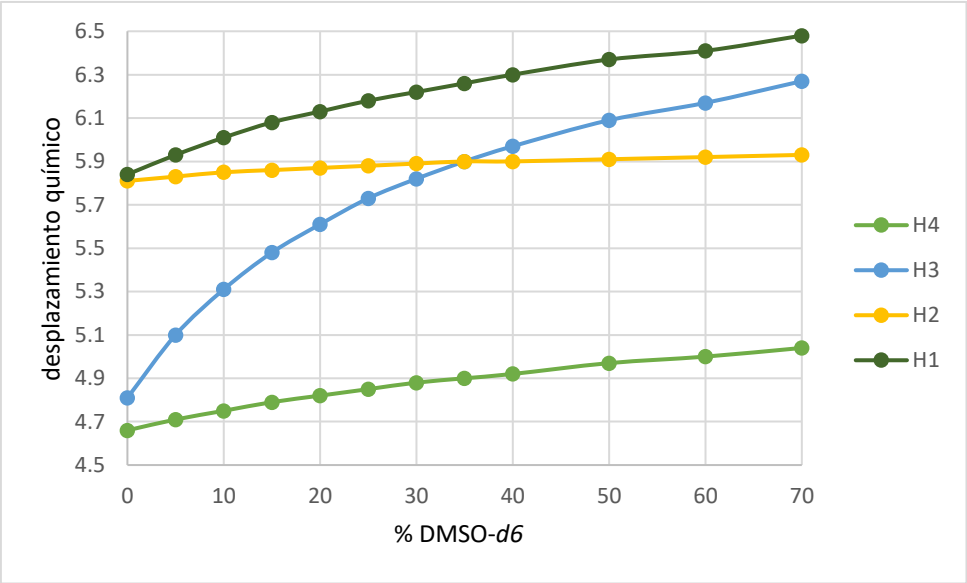


Figure 8. DMSO-d6 titration of a 2 µM sample of **3** at +25 °C ($CDCl_3$, 500 MHz).

As can be seen in Figure 8, which plots the chemical shift value of the HN protons of **3** with respect to the ratio of DMSO-*d6*, as DMSO-*d6* is added, H3 undergoes a large shift at low field, whereas for H4 and H1 the shift is much milder and practically non-existent for H2. These results suggest that H2 is less accessible to hydrogen bond formation with DMSO-*d6*, which would support it being part of intramolecular hydrogen bonds. The large variation of the δ of H3 with the addition of DMSO-*d6* is consistent with this HN being exposed or accessible to the solvent and therefore also accessible to DMSO-*d6*. The H_1 and H_4 protons are in an intermediate situation, possibly also forming intramolecular hydrogen bonds.

- NOESY

To confirm the above indications of the presence of intramolecular hydrogen bonds in our compound and thus of a β-turn secondary structure, 2D-NOESY spectra were analyzed.

The experiments were performed in $CDCl_3$ and the parameters used were: 500 Mz, a mixing time of 600 ms, a concentration of 5 mM and +25 °C. The results are shown in Figures 9 and 10.

No long range (LR = "Long Range") contacts are observed between the two ends of the hairpin (residues Bn and Et), which could justify the existence of a β-turn. The only NOE contacts observed are short-range:

- H2/H4 and H_2/CH_2 NOE of the ethyl chain.
- Sequential H2/CH2 exocyclic NOE of the Cy unit.

Chemical exchange (EXSY) is also observed between HNs and solvent water (δ = 1.6 ppm), except H1.

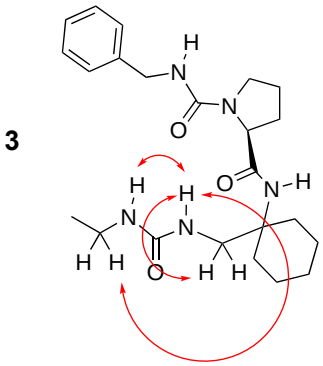


Figure 9. Summary of NOE of compound **3** at +25 °C, t-mix = 600 ms ($CDCl_3$, 500 MHz).

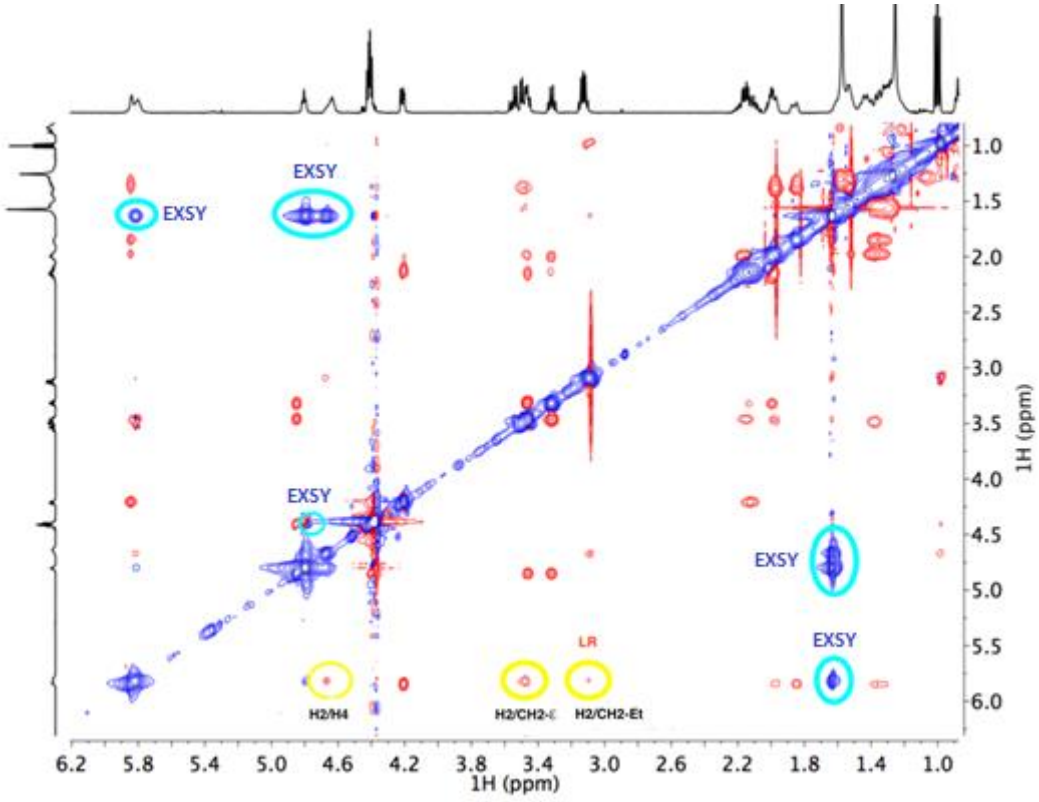


Figure 10. 2D NOESY spectrum of compound 3 at +25 °C, t-mix = 600 ms ($CDCl_3$, 500 MHz). Sign of NOE peaks; red negative, blue positive.

Given the results obtained, which do not support the presence of a β-turn conformation, it was decided to repeat the NOESY experiment by lowering the temperature to -10 ºC, in order to reduce the exchange rate of the HNs, and to reduce the rate of conformational exchange between the β-turns (if any) and other extended conformations. The experiment (Figures 11 and 12) was successful and allowed confirmation of the β-turn ("hairpin") conformation. This confirmation is based on the observation of long-range contacts, namely NOE was observed between:

- The aromatic protons of phenyl/CH_3 of ethyl urea.
- Benzyl CH2/CH3 of ethyl urea.
- Benzyl CH2/H4.

Comparison of NOE at −10 °C and +25 °C suggests a rapid conformational equilibrium between the β-turn and other extended conformations.

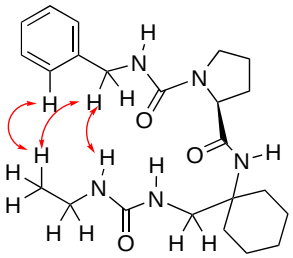


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Figure 11. Summary of NOE of compound 3 at −10 °C, t-mix = 600 ms ($CDCl_3$, 500 MHz).

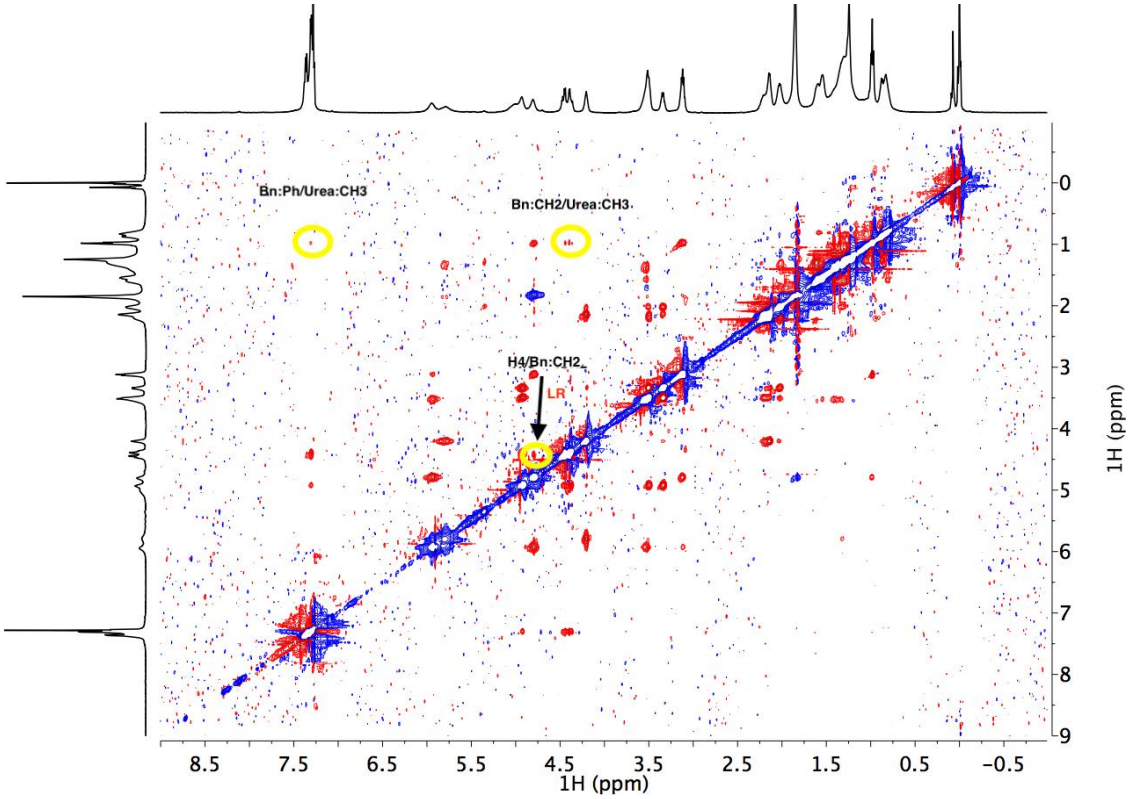

Figure 12. 2D NOESY spectrum of compound **3** at −10 °C, t-mix = 600 ms ($CDCl_3$, 500 MHz). Sign of NOE peaks; red negative, blue positive.

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