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A Nonpeptidic Reverse-Turn Scaffold Stabilized by Urea-Based Dual Intramolecular Hydrogen Bonding

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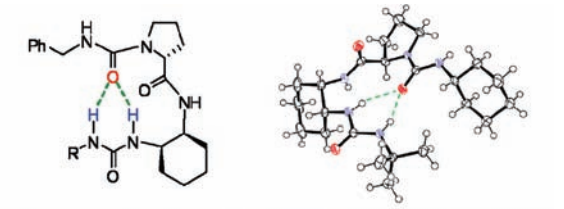
ABSTRACT



D-Pro-DACH/Urea: Reverse-turn scaffold stablized by dual H-bonding

A novel nonpeptidic reverse-turn scaffold containing urea fragments that are connected by a conformationally constrained D-prolyl-*cis*-1,2-diaminocyclohexane (D-Pro-DACH) linker is reported. The scaffold adopts a well-defined reverse-turn conformation that is stabilized by dual intramolecular hydrogen bonding in both solution and solid states.

Reverse turns are found abundantly in biologically active peptides and globular proteins. They serve as molecular recognition sites in many biological events due to their potent binding and exquisite selectivity.[1] Numerous efforts have been made to study reverse-turn scaffolds in order to develop potent therapeutic agents[2] and to identify loop segments that can induce autonomous β-sheet folding.[3] In addition, it has been demonstrated that short-length peptide fragments containing reverse turns showed excellent catalytic activity for asymmetric organic transformations.[4] However, the use of peptidic reverse turns for these applications is often limited because natural peptides are not resistant to enzymatic degradation and are conformationally flexible in solution. Although many examples of nonpeptidic reverse-turn surrogates have been reported to overcome the drawbacks,[5] it is still challenging to find a new type of nonpeptidic scaffold that can adopt a highly populated reverse-turn conformation in solution. Herein, we report a novel nonpeptidic reverse-turn scaffold, which contains two urea fragments linked by a conformationally

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constrained D-prolyl-*cis*-1,2-diaminocyclohexane (D-Pro-DACH). The scaffold was envisioned to adopt a reverse-turn conformation via dual intramolecular hydrogen bonding in both solution and solid states.

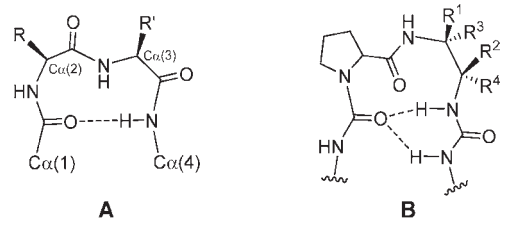
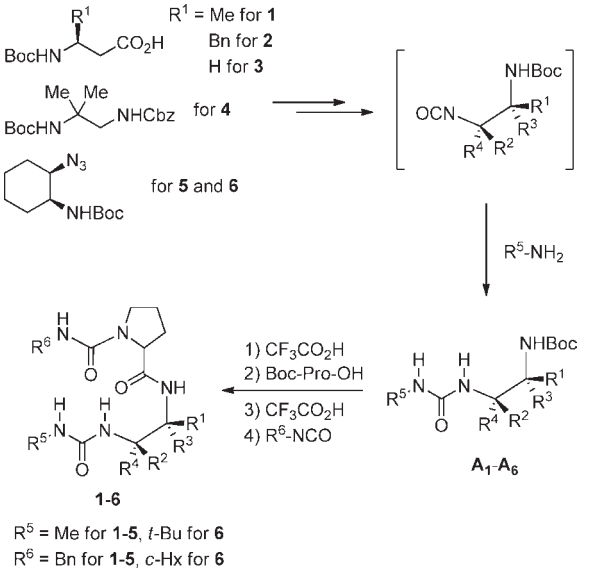


Figure 1. Conventional peptide reverse-turn (β-turn) structure (left) and nonpeptidic reverse-turn scaffold stabilized by urea-based dual intramolecular hydrogen bonds (right).

Conventional reverse-turns (β-turns) are composed of tetrapeptide sequences, in which the $C\alpha_{(1)}$—$C\alpha_{(4)}$ distance is ≤ 7 Å, mostly with hydrogen bonding between $C{=}O_{(1)}$ and $N{-}H_{(4)}$ that stabilizes the well-defined conformation (Figure 1).[6] To ensure a conformationally stable nonpeptidic reverse-turn scaffold, we employed urea functionality to introduce simultaneous formation of 10- and 12-membered C=O---H—N hydrogen bonds.[7] Due to the dual hydrogen bonding, the scaffold **B** was expected to adopt a more stable reverse-turn conformation, being populated predominantly in solution, than the amide version **A**. In this design, we chose a prolyl-1,2-diamine linker to connect two urea functionalities.[7b] To find the best combination between D-/L-proline and 1,2-diamine derivatives, the substitution pattern on 1,2-diamine was systematically screened from the unsubstituted one to the conformationally rigid *cis*-1,2-DACH.

We prepared a series of reverse-turn candidates as summarized in Scheme 1. Each starting material was converted to the corresponding isocyanate which was then treated with primary amines (CH_3NH_2 for **1**–**5** and *t*-BuNH_2 for **6**; see Supporting Information (SI) for details) to synthesize the bottom fragments $\mathbf{A_1}$–$\mathbf{A_6}$. After removal of Boc followed by coupling with Boc-Pro-OH, the resulting amides were sequentially treated with TFA and isocyanates (benzyl for **1**–**5** and cyclohexyl for **6**) to obtain the desired turn candidates. Reference compounds, **7** (an amide version of **1**), **8a**,[8] and **8b**[9] (upper and bottom fragments), were also prepared (see SI).

Scheme 1. Summary of the Synthetic Procedures of Nonpeptidic Reverse-Turn Candidates (**1**–**6**)[a]



[a] All substituents (R^1, R^2, R^3, R^4) and the stereochemistries of prolines are given in Table 1.

To evaluate the extent of the hydrogen bonding in **1**–**6**, we determined, at first, the temperature coefficients ($-\Delta\delta/\Delta T$) for the four NH protons by measuring their chemical shifts in DMSO-d_6 at temperatures ranging from 298 to 348 K (Table 1).[10] In all reverse-turn candidates **1**–**6**, the values for $NH_{(3)}$ and $NH_{(4)}$ ($-\Delta\delta/\Delta T < 5$) were much smaller than those for $NH_{(1)}$ and $NH_{(2)}$ ($-\Delta\delta/\Delta T \geq 5$), whereas the values for the three NHs in the reference amide **7** were >5 with $NH_{(2)}$ (5.4) $\approx$ $NH_{(3)}$ (5.5). The results indicate that $NH_{(1)}$ and $NH_{(2)}$ are in solvent accessible positions, and $NH_{(3)}$ and $NH_{(4)}$ are participating in intramolecular hydrogen bonding.[10] Notably, the values were not strongly influenced by the stereogenic center of proline (**1a** vs **1b** and **2a** vs **2b**), which is different from other reverse-turn motifs such as Pro-DADME;[11] thus we considered only the D-Pro series for further studies.

For $NH_{(4)}$, a significant change of the coefficients (0.6–4.5) was observed upon variation of the substitution pattern in the diamine linkers. A trend was seen that the value increases as the flexibility of the diamine linker increases: **5** (0.6) or **6** (1.6) < **4** (2.5) < **2a** (2.9) < **1a** (3.1) < **3** (4.5). The combination of the urea functionality with a conformationally constrained linker resulted in remarkably low temperature coefficients for the $NH_{(4)}$ in **5** or **6**. The data also show a nonequivalent hydrogen bonding pattern between $NH_{(3)}$ and $NH_{(4)}$, in which the

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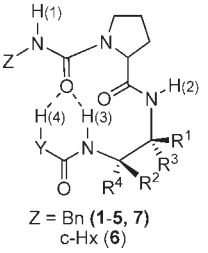
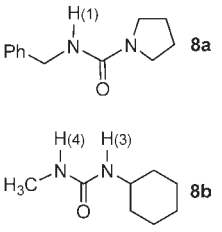
Table 1. Temperature Coefficients of **1**–**7** and Chemical Shifts of Selected Compounds





		substituents					temperature coefficients[a]				chemical shifts (ppm)[b,c]			
	Pro	R^1	R^2	R^3	R^4	Y	$NH_{(1)}$	$NH_{(2)}$	$NH_{(3)}$	$NH_{(4)}$	$NH_{(1)}$	$NH_{(2)}$	$NH_{(3)}$	$NH_{(4)}$
1a	D-	CH_3	H	H	H	NCH_3	7.5	5.2	3.9	3.1				
1b	L-	CH_3	H	H	H	NCH_3	7.0	5.1	3.4	3.5				
2a	D-	CH_2Ph	H	H	H	NCH_3	7.1	5.1	3.2	2.9				
2b	L-	CH_2Ph	H	H	H	NCH_3	7.8	5.3	3.8	2.8				
3	D-	H	H	H	H	NCH_3	7.8	5.9	4.3	4.5	4.80	6.94	5.18	4.45
4	D-	CH_3	H	CH_3	H	NCH_3	8.1	5.8	4.6	2.5	4.77 (2.01)	6.00 (1.59)	5.71 (0.37)	4.53 (1.10)
5	D-	$-(CH_2)_4-$		H	H	NCH_3	8.1	6.6	4.7	0.6	4.72 (2.21)	6.51 (1.10)	5.49 (0.34)	4.63 (0.82)
6	D-	$-(CH_2)_4-$		H	H	N-^{t}Bu	6.3	5.0	3.8	1.6	4.20 (1.60)	6.56 (0.85)	5.31 (0.37)	4.30 (1.07)
7	D-	CH_3	H	H	H	CH_2	7.1	5.4	5.5					
8a											4.43 (2.19)			
8b													4.08 (1.67)	4.08 (1.50)

[a] Temperature coefficients ($-\Delta\delta/\Delta T$) were measured in DMSO-d_6. [b] Chemical shifts of the amide NHs and urea NHs of **3**–**6** and **8a**,**b** (2 mM in $CDCl_3$ at room temperature). [c] Values in parentheses denote $\Delta\delta$ (ppm) resulted from DMSO-titration experiments at 298 K.

latter is more sensitive to the linker flexibility than the former.

The evidence of dual intramolecular hydrogen bonding was strengthened by the DMSO-titration experiment.[7c] For example, the chemical shifts of $NH_{(3)}$ and $NH_{(4)}$ in **5**, appearing more downfield than those in reference compound **8b** under nonaggregating conditions (2 mM in $CDCl_3$ at room temperature),[12] were changed less significantly than those of $NH_{(1)}$ and $NH_{(2)}$ in **5** or NHs in reference compounds when DMSO-d_6 was added gradually to the $CDCl_3$ solution (Table 1). This result suggests that both $NH_{(3)}$ and $NH_{(4)}$ are involved in intramolecular hydrogen bonding. Both the temperature coefficient study and DMSO-titration experiment indicated the existence of dual intramolecular hydrogen bonding that is mostly stable in **5**.

To confirm the reverse-turn conformation of the scaffold in both solution and solid state, we carried out NMR and X-ray crystallographic studies. For the solution state conformation, NOESY experiments for compounds **3**–**6** in $CDCl_3$ at 4 °C were performed after all the resonances were assigned by COSY and TOCSY. The result for compound **5**, for example, is summarized in Figure 2. Two NOE signals for $NH_{(1)}-H_{(11)}$ and $NH_{(3)}-NH_{(4)}$ indicate that both urea fragments are in a *trans*–*trans* conformation and, thus, the NHs are oriented opposite to their urea carbonyl groups. A signal between $H_{(14)}$ and $NH_{(2)}$ signifies the two urea fragments facing each other for a proper alignment of hydrogen bonding donors ($NH_{(3)}$ and $NH_{(4)}$) and acceptor ($C_{(9)}$=O). The correlation between the $H_{(17)}$ and $H_{(22)}$ indicates the role of the *cis*-cyclohexyldiamine ring, providing an appropriate torsional angle for the reverse-turn conformation. Furthermore, the nonadjacent correlation between $NH_{(4)}$ and $H_{(7)}$ unambiguously defines the stable turn conformation.[13] In

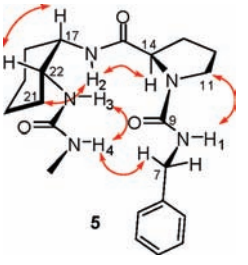


Figure 2. Summary of NOE signals for **5** in $CDCl_3$ (400 MHz, mixing time 300 ms, in 2 mM solutions) at 4 °C.

(12) The plot of concentration vs chemical shift for compound **5** showed that the concentration of 7.78 mM was the nonaggregation limit, so 2 mM was chosen for the NMR studies.

a variational temperature study (from 323 to 223 K), both $NH_{(3)}$ and $NH_{(4)}$ resonances were gradually shifted to downfield as temperature went down, which supports further that **5** adopts a stable reverse-turn conformation via dual intramolecular hydrogen bonding (see SI). The overall results from the NMR studies revealed that the designed reverse-turn scaffold **5** is conformationally stable in the solution state.

For the solid state confomational analysis of the turn scaffold, compound **6**,[14] which showed similar temperature coefficients and chemical shifts to those of **5**, was crystallized from a mixture of EtOH−CH_2Cl_2 (1:4) by slow evaporation over a period of two days. The X-ray crystallographic analysis apparently revealed the existence of dual intramolecular hydrogen bonding ($d_{N(1)\cdots O(3)}$ = 3.042 Å, $\angle_{N(1)H\cdots O(3)}$ = 157.5° and $d_{N(2)H\cdots O(3)}$ = 3.022 Å, $\angle_{N(2)H\cdots O(3)}$ = 159.6°) (Figure 3).[15,16] The backbone torsion angles χ_1 ($C_{(17)}-N_{(4)}-C_{(13)}-C_{(12)}$) and χ_2 ($N_{(3)}-C_{(11)}-C_{(6)}-N_{(2)}$) are +61.50° and +56.42°, respectively.[17] The two urea groups are slightly twisted by 17.7°, and the central amide group, $HN_{(3)}-C{=}O_{(2)}$, is nearly perpendicular to the mean plane composed of four urea nitrogens (79.24°). Additionally, the distance of 5.02 Å between $C_{(4)}$ and $C_{(18)}$ is within the criterion of a reverse turn (7 Å). This result indicates that the scaffold is well adopted in a reverse-turn conformation via 10,12-membered dual intramolecular hydrogen bonding in the solid state,[18] as in the solution state.[19]

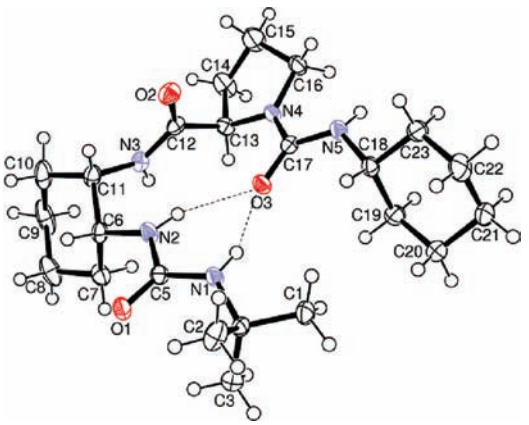


Figure 3. Crystal structure of **6**.[16] H-bonds shown as dashed lines.

In summary, we have developed a novel nonpeptidic reverse-turn scaffold containing urea fragments that are connected by a conformationally constrained D-prolyl-*cis*-1,2-diaminocyclohexane (D-Pro-DACH) linker. Owing to the dual intramolecular hydrogen bonding, the scaffold adopts a well-defined reverse-turn conformation, which is more stable than its amide analogue, in both solution and solid states. Our reverse-turn motif could serve as an N-terminus-to-N-terminus linker for developing nonpeptidic parallel β-sheets and new reverse-turn mimetics.

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Supporting Information Available. Experimental details, characterization of all new compounds, ^1H and ^{13}C NMR spectra of **1**−**8**, IR spetra of **6**, NOESY spectra of **5**, computational analysis, and CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

(13) The NOE correlation was also observed in aqueous DMSO-d_6 (see SI), whereas the signal for **2b** at 5 mM in $CDCl_3$ was not seen.

(14) The high crystalline nature of cyclohexyl and *tert*-butyl substituents in β N−O turns and helices was reported: Yang, D.; Zhang, Y.-H.; Zhu, N.-Y. *J. Am. Chem. Soc.* **2002**, *124*, 9966.

(15) Crystal data for **6**: $C_{23}H_{41}N_5O_3$, M_w = 435.32, monoclinic, space group *P*2(1), *a* = 10.1633(2) Å, *b* = 9.9300(2) Å, *c* = 12.1706(3) Å, α = 90.00°, β = 96.2770(10)°, γ = 90.00°, *V* = 1220.91(5) Å^3, Z = 2, ρ_{calcd} = 1.185 g cm^{-3}, μ = 0.080 min^{-1}, *T* = 130 K, 12 037 reflections measured, independent: 5837 (R_{int} = 0.017). The structure was solved by direct methods and refined by full-matrix least-squares on F^2; R_1 = 0.0357, wR_2 = 0.0904 [$I > 2\sigma(I)$]; Flack(*x*) parameter = 0.0(8) maximal residual electron density: 0.47 eÅ^{-3}. CCDC 730130.

(16) The compound numbering is arbitrary and different from those in the NMR structure.

(17) Other dihedral angles in **6** such as ϕ_{i+2} and ψ_{i+2} that can be seen in a natural β-turn are not analyzed since *cis*-1,2-DACH is not originated from a natural amino acid as per the usual nomenclature convention: ref 7c and references therein.

(18) For a recent example of an intramolecular H-bond for turn mimics in the solid state, see: (a) Sacchetti, A.; Silvani, A.; Lesma, G.; Pilati, T. *J. Org. Chem.* **2011**, *76*, 833. (b) Lesma, G.; Landoni, N.; Pilati, T.; Sacchetti, A.; Silvani, A. *J. Org. Chem.* **2009**, *74*, 8098.

(19) An IR study of **6** also supported the existence of the hydrogen bonding (see SI). In addition, the computational analysis is consistent with the experimental results (see SI).