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Imidazoline Pseudodipeptides as Mimics of Reverse Turn Structures

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Abstract: The synthesis of an imidazoline dipeptide mimetic (a 4,5-dihydroimidazole-4-carboxylic acid) is reported that displays an intramolecular hydrogen-bond consistent with a turn conformation in solution.

Keywords: imidazolines; pseudodipeptides; reverse turns

Reverse turns (including β-turns) **1** (Figure 1) are significant elements in protein conformation, and are often associated with regions of a protein sequence associated with biological activity. For this reason, many molecules have been designed and synthesized as mimics of the β-turn sub-unit structure [1,2]. The four-residue set of amino acids (i to $i+3$) that constitute a reverse turn is characterized by a hydrogen bond between the amide carbonyl oxygen of residue i and the NH hydrogen of residue $i+3$, and often contains the cyclic amino acid proline at residues $i+1$ or $i+2$, and a glycine residue. Specific types of β-turn have been classified [3]. As part of a programme of synthesis of heterocycles, a peptide bond replacements in pseudopeptides having restricted conformations [4], we have reported the cyclic amidines **2** as pseudodipeptides (Figure 2) [5–7]. Their relationship with cyclic amino acids led us to propose suitable derivatives **3** of the imidazolines **2** containing the backbone framework necessary to form the same 10-membered ring hydrogen-bonded framework as reverse turn **1**, to examine their potential for enforcing turn structures on (peptide) chain segments. We report here the preparation of two glycyl pseudopeptides **3** and nuclear magnetic resonance (NMR) studies that show intramolecular hydrogen-bonding.

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Our retrosynthetic analysis of **3** proposed a glycine-based imidate and a 2,3-diaminopropanamide as precursors (Scheme 1). Initial target **3a** was, therefore, assembled from aminoacetonitrile hydrogen sulphate, which was acylated with 4-bromobenzoyl chloride (toluene, aq. NaOH, 0°C) to give the nitrile **4** (88%), Scheme 2. This was converted to the imidate salt **5** (87%) using a Pinner reaction (2 mol equiv. EtOH, CH_3COCl, 0°C). The imidate was not stored, but reacted directly with the anilide **6a** of 2,3-diaminopropanoic acid. This was prepared by treatment of (*R*,S)-2,3-diaminopropanoic acid[1] with benzyl chloroformate (aq. NaOH, 0°C) to afford the *bis*-carbamate **7**. Coupling with aniline was accomplished *via* the pentafluorophenyl ester; reaction of **7** with pentafluorophenol (DCCI, CH_2Cl_2, 0°C) gave the active ester (69% after column chromatography[2]) that was treated with aniline (CH_2Cl_2, 20°C) to afford amide **8a** (86%). Finally, deprotection of *bis*-carbamate **8a** by hydrogenolysis (H_2, $Pd(OH)_2$-C, MeOH, 20°C) afforded the *N*-phenyl-2,3-diaminopropanamide **6a** (quantitative). Condensation of this diamine with the imidate **5** (MeOH, reflux) produced the first target imidazoline **3a** (64%).

We had hoped that the diaryl derivative **3a** would allow investigation in the solid state *via* an X-ray crystal structure, but it did not prove possible to obtain suitable crystals. Studies to probe for

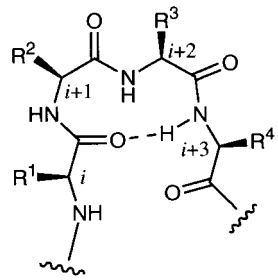


Figure 1 A peptide reverse turn.

intramolecular hydrogen-bonds in solution as an indicator of a turn conformation, have used measurement of amide NH chemical shifts and their changes with temperature in the ^{1}H-NMR spectrum in non-polar solvents [8,9], but the low solubility of **3a** in CH_2Cl_2 or $CHCl_3$ precluded such experiments. Amide **3a** is soluble in dimethylsulfoxide (DMSO), but although conformational studies on β-turn mimics have been reported in this solvent [10], it was felt prudent to avoid this hydrogen-bond acceptor solvent, capable of disrupting any intramolecular hydrogen-bond. We thus proceeded to modify our target imidazoline to improve $CHCl_3$ solubility.

This was achieved by replacement of the phenyl amide by a butyl amide, which was easily accomplished by coupling of the *bis*-carbamate **7** with butylamine. The pentafluorophenyl ester of acid **7** was again employed, and its reaction with butylamine gave the protected *N*-butylamide **8b** (76%). Hydrogenolysis as before led to the *N*-butyl-2,3-diaminopropanamide **6b** (88%), and condensation of this diamine with the imidate **5** (MeOH, reflux) produced the second generation target imidazoline **3b** (47%, unoptimized). In an alternative approach to more hydrophobic imidazolines, and for comparison purposes, the carbamates **9a,b** were prepared from the anilide **3a** (for **9a**: Boc_2O, $NaHCO_3$, 1:1 THF:H_2O v/v, 20°C; 65%; for **9b**: CH_3OCOCl, $NaHCO_3$, 1:1 THF:H_2O v/v, 20°C; 60%) (Figure 3).[3]

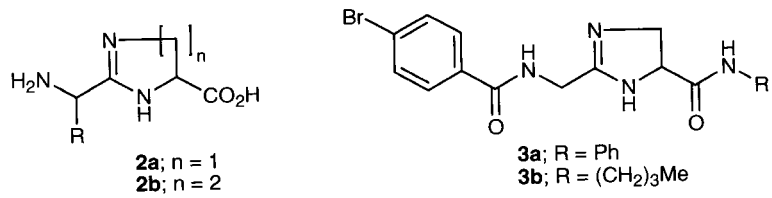
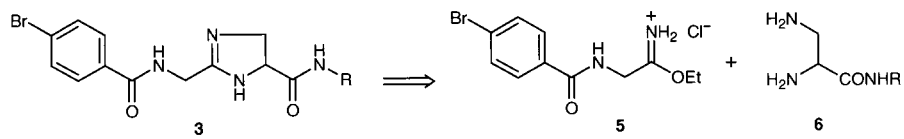
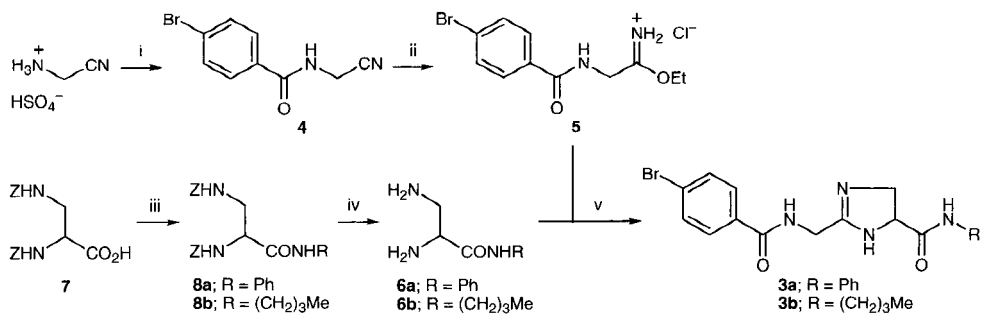


Figure 2 Cyclic amidine pseudodipeptides.



Scheme 1 Retrosynthetic analysis of pseudodipeptides **3**.



Reagents: i, 4-bromobenzoyl chloride, toluene, aq. NaOH, 0°C; ii, 2 EtOH, CH_3COCl, 0°C; iii, pentafluorophenol, DCC, CH_2Cl_2, 0°C; $PhNH_2$ (for **8a**) or $Me(CH_2)_3NH_2$ (for **8b**), CH_2Cl_2, 20°C; iv, H_2, $Pd(OH)_2$-C, MeOH, 20°C; v, MeOH, reflux

Scheme 2 Synthesis of pseudopeptides **3**.

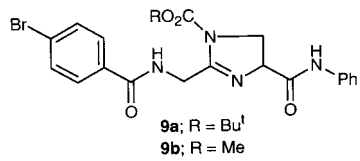


Figure 3 Carbamoylated pseudodipeptides **9**.

Whilst the additional hydrophobic substituent in **3b** hindered crystallinity, ^{1}H-NMR spectroscopy in non-polar solvent was now possible. At room temperature in $CDCl_3$, the two amide NH protons of **3b** were distinguished by a ^{1}H-^{1}H correlated spectroscopy (COSY) experiment. The butylamide proton is observed at δ 6.6–6.7, slightly upfield of typical shifts for intramolecularly hydrogen-bonded NH protons (δ 7–8), but downfield of those of free NH protons (δ 6). Variable temperature studies over the range 273–323 K, and at low concentration (5 mM) to minimize intermolecular interactions, revealed a very small change of chemical shift with temperature for the butylamide proton, of -1.84 ppb K^{-1} (Figure 4). It is well documented that changes in temperature have little effect on the chemical shifts of protons involved in an intramolecular hydrogen-bond, or otherwise shielded from the medium. Exposed hydrogens, those which are accessible to the solvent, exhibit a larger temperature coefficient (>4 ppb K^{-1}) than do intramolecularly hydrogen-bonded hydrogens (<3 ppb K^{-1}). The value recorded for imidazoline **3b** is thus well below the cut-off implying that it is sequestered from solvent and intramolecularly hydrogen-bonded in $CDCl_3$.

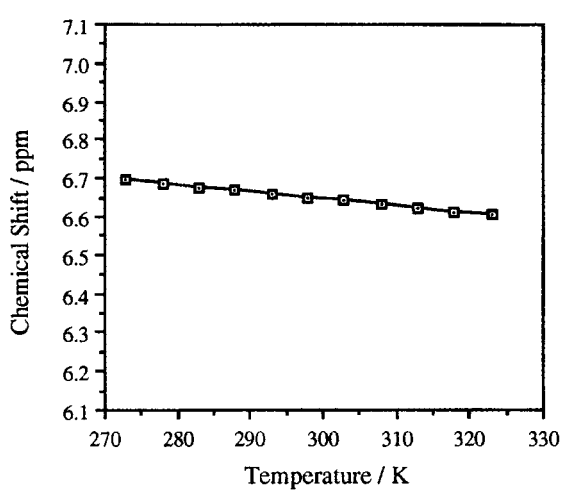


Figure 4 Variation with temperature of the butylamide NH chemical shift for imidazoline **3b**.

In contrast, the carbamates **9a,b** had anilide NH proton resonances in the range δ 8.4–9.0, but with temperature coefficients of -8.27 and -9.46 ppb K^{-1}, respectively, effectively excluding the possibility of an intramolecular hydrogen-bond. Infrared (IR) spectra of **3b** in dilute $CDCl_3$ solution (1 mM) reveal two strong overlapping peaks, at 3450 cm^{-1} and 3406 cm^{-1}, which can be assigned to non-hydrogen-bonded NH and a weakly intramolecularly hydrogen-bonded NH, respectively.

We have thus shown that the imidazoline pseudodipeptide **3b** displays an intramolecular hydrogen-bond consistent with adoption of a turn-like conformation. Studies to refine this conformational constraint are under way.

Acknowledgements

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NOTES

1. No other chiral centres were to be present in compounds **3**, hence no problems with diastereoisomers would arise, so use of racemic diaminopropanoic acid was considered acceptable.
2. Purification of the pentafluorophenyl ester, to remove contaminant dicyclohexylurea (DCHU), proved necessary for efficient formation of clean amide.
3. The carbamates **9** are drawn (as we have done elsewhere, [5]) as N – 1 substituted on steric grounds, although definitive location must await further evidence.

REFERENCES

1. Kahn M (ed.). Peptide secondary structure mimetics. Symposia-in-Print No. 50. *Tetrahedron* 1993; **49**: 3433.
2. Hanessian S, McNaughton-Smith G, Lombart HG, Lubell WD. Design and synthesis of conformationally constrained amino acids as versatile scaffolds and peptide mimetics. *Tetrahedron* 1997; **53**: 12 789–12 854.
3. Ball JB, Hughes RA, Alewood PF, Andrews PR. Turn topography. *Tetrahedron* 1993; **49**: 3467–3478.
4. Jones RCF, Hollis SJ, Iley JN. 3-(1-Aminoalkyl)isoxazole-4-carboxylic acids as peptide bond replacements. *Tetrahedron: Asymmetry* 2000; **11**: 3273–3276.

5. Gilbert IH, Rees DC, Crockett AK, Jones RCF. Imidazolines as amide bond replacements. *Tetrahedron* 1995; **51**: 6315–6336.
6. Jones RCF, Crockett AK. The synthesis of unusual tetrahydropyrimidine amino-acids. *Tetrahedron Lett*. 1993; **34**: 7459–7462.
7. Jones RCF, Dickson J. An imidazoline pseudodipeptide suitable for solid phase peptide synthesis. *J. Peptide Sci*. 2000; **6**: 621–624.
8. Huck BR, Fisk JD, Gellman SH. Promotion of sheet formation in α-peptide strands by a β-peptide reverse turn (for leading references). *Org. Lett*. 2000; **2**: 2607–2610.
9. Fisk JD, Powell DR, Gellman SH. Control of hairpin formation *via* proline configuration in parallel β-sheet model systems (for leading references). *J. Am. Chem. Soc*. 2000; **122**: 5443–5447.
10. Dumas J-P, Germanas JP. Design, synthesis and evaluation of a novel bicyclic lactam as a Gly-Pro type-VI β-turn mimic (for example). *Tetrahedron Lett*. 1994; **35**: 1493–1496.

