ChemPubSoc Europe

DOI: 10.1002/chem.201503062

CHEMISTRY
A European Journal
Communication

Biomimetic Synthesis

Design, Synthesis, and Conformational Analysis of Proposed β-Turn Mimics from Isoxazoline-Cyclopentane Aminols

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Dedicated to Irene Lucia Novara and Peppino Quadrelli on the occasion of their 85th birthday

Abstract: Constrained aminols from oxazanorbornene derivatives have the geometrical features to be used as β-turn inducers. Four different stereoisomers were prepared and spectroscopically characterized (MD calculations, NMR-titration and VT-NMR experiments). Temperature coefficients in DMSO are indicative for the existence of an intramolecular hydrogen bond. Chirooptical properties revealed a β-turn arrangement of all the synthesized compounds, where, depending on the absolute configuration of the cyclopentane spacer, they can be labeled as left- or right-handed turns.

In the regulation of nearly every biological process, a pivotal role is dictated by peptide ligands and protein receptors.[1] Besides the understanding of the bioactivity, three-dimensional interactions are essential for the design of analogues during the drug discovery process. The determination of the spatial orientation deeply involves the conformational analysis on the basis of spectroscopic techniques and molecular modelling.[2] As far as flexible molecules are concerned, the key point is the correspondence among conformation found in solution, real physical meaning, and in-silico determined conformations.[3] Conformationally constrained molecules are the probes to get information about the three-dimensional interplay between ligand and receptor. Many efforts have been made in this area[4] to mimic secondary structural features and β-turns are the most common observed in small peptides and proteins.[2] Turns are essential for protein structure and also occur within protein binding sites, at protein–protein interfaces and in small bioactive peptides, playing a crucial role in recognition.[5] Most β-turns contain an intramolecular hydrogen bond between the carbonyl oxygen of the first residue (*i*) and the amide NH proton of the fourth residue ($i+3$), forming a pseudo-ten-membered ring (Figure 1).[6]

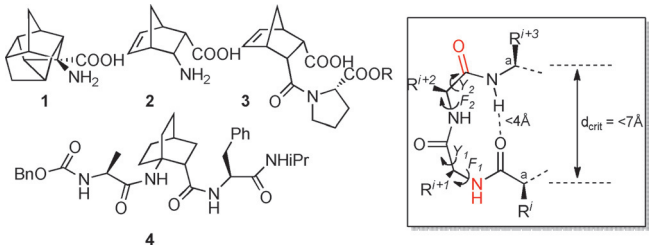

Figure 1. Compounds and products for turn induction. In the inset: criteria for the identification of β-turns.

Critical distances are defined between the Cα carbon atoms, which must be <7 Å. A second requirement is the distance between the carbonyl oxygen of the first amino acid and the amide hydrogen of the fourth one, which must be <4 Å. In general, the use of cage amino acids allows for the induction of a β-turn by the complete replacement of the three amino acids required normally.[7–9]

The synthesis of analogues designed to stabilize a peptide chain is constantly pursued and examples of cyclic and bicyclic modifications have been recently reported in literature,[9a] for example, spirolactam-bicyclic and tricyclic proline based systems,[10] medium-ring heterocyclic compounds[11] and conformationally constrained β-amino acids.[12]

Cage α-amino acids of type **1** incorporate a bulky hydrophobic disubstituted cage while β-amino acids of type **2** find in the norbornene skeleton the best residue to generate a turn-inducer.[7] Non-racemic amides **3** were the key step for a successful application of this type of chemistry towards metalloprotease inhibitors. The use of peptidic reverse turns is often limited because natural peptides are not resistant to enzymatic degradation. Nonpeptidic (*S*)-aminobicyclo[2.2.2] octane-2-carboxylic acid **4** (H-(*S*)-ABOC-OH) were reported as reverse turns into peptides.[5]

Recently we have proposed the synthesis of a new class of cyclopentane aminols starting from cyclopentadiene using the nitrosocarbonyl intermediates (RCONO, **5**) chemistry.[13]

These intermediates, generated by the mild oxidation of nitrile oxides with tertiary amine *N*-oxides or by oxidation of hydroxamic acids, are efficiently trapped by cyclopentadiene (Scheme 1) to afford the hetero Diels–Alder (HDA) cycloadducts **6**, highly reactive dipolarophiles employed to synthesize the conformationally restricted carbocyclic aminols **8** through amide hydrolysis and N–O bond cleavage of the cycloadducts **7**.[7,14]

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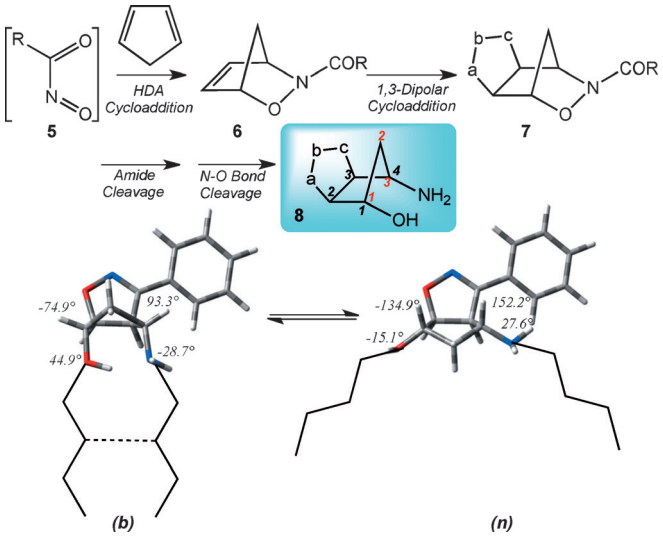
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201503062.



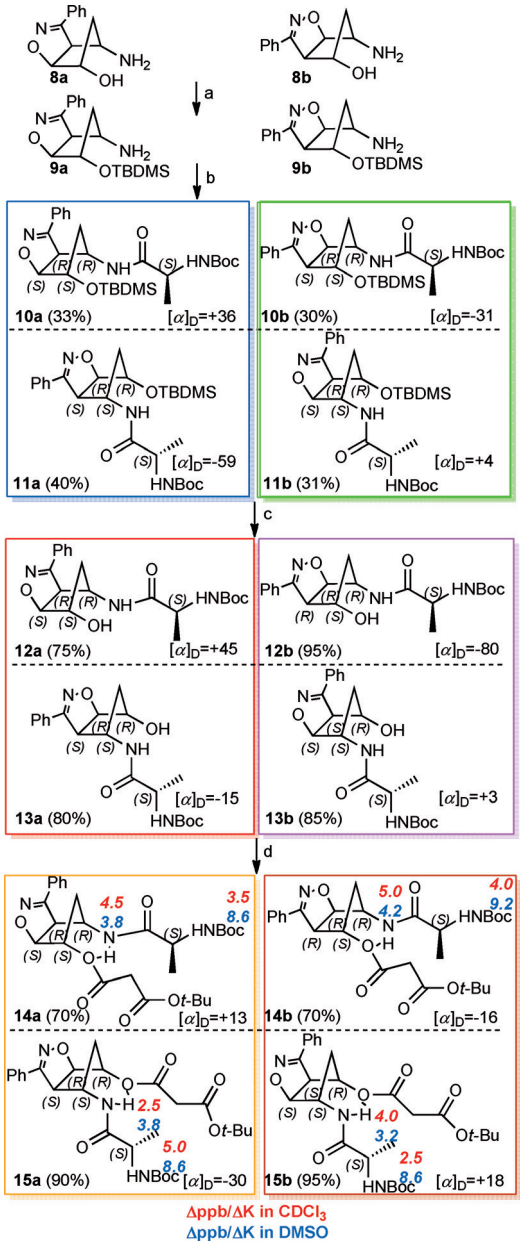
Scheme 1. Synthetic pathway of isoxazoline-cyclopentane aminols through nitrosocarbonyl chemistry. Boat- and chair-like conformations of the aminol **8**. Side chain structures are shown as hydrogen-bonded (*b*) and non-hydrogen-bonded (*n*).

We wish to present here our conceptual approach towards the preliminary design of original turn mimics. Boat- and chair-like conformations are possible for the aminolic structures (calculated in the gas phase at the DFT B3LYP/6-31+G(d,p) level);[15] the aim is to ascertain whether adequate elongation from the hydroxy and amino groups with side chains incorporating aminoacidic residues can provide a stabilized turn structure (Scheme 1) of type hydrogen-bonded (*b*) or non-hydrogen-bonded (*n*).[16]

The regioisomeric aminols **8a,b**, prepared according to a previously reported methodology,[7,14] nicely resemble the structures of external mimetics because of the reduced conformational flexibility determined by the isoxazoline ring fused to the cyclopentane moiety.[17] To develop our concept, the synthetic strategy for the preparation of depsipeptide-like structures is outlined in Scheme 2.

The protection of the hydroxy group was performed under standard procedure affording the amines **9a,b** in 85% each. Compounds **9a,b** were coupled with the commercially available L-Alalnine *N*-Boc protected. Diastereoisomers **10a** and **11a** were isolated in good yields and similarly diastereoisomers **10b** and **11b** obtained from **9b**. The deprotection of the hydroxy functionalities was secured by standard nBu$_4$NF treatment. The alcohols of type **12a,b**/**13a,b** were obtained in very good yields.

The alcoholic groups of the compounds **12** and **13** were then derivatized as esters of a desimmetrized malonic acid easily prepared upon treatment with malonic acid with anhydrous pyridine in *ter*-butanol and adding dropwise an equimolecular amount of methanesulfonyl chloride.[18] This choice was done to avoid the presence of a further stereogenic center and to terminate the elongation with a carboxylic moiety facing the amino group on the opposite chain. The esterification was conducted with a typical DIC/DMAP coupling procedure in DCM at room temperature for 48 h. The ester derivatives **14a/15a** and **14b/15b** were obtained from good to optimum yields.



Scheme 2. Synthesis of the turn mimic compounds: a) TBDMSiCl, Imidazole, CH_2Cl_2, RT, 18 h. b) *N*-Boc-L-Ala, HBTU, DIEA, CH_2Cl_2, RT, 48 h. c) nBu$_4$NF, THF, RT, 3 h. d) HOOC-CH_2-COO*t*Bu, DIC, DMAP, CH_2Cl_2, RT, 48 h. Temperature coefficients of amide and carbamate protons of **14a,b** and **15a,b** in $CDCl_3$ (298.15–318.15 K) and DMSO (298.15–348.15 K). Yields and $[\alpha]_D$ (C=1, MeOH) are also reported.

The conformational analysis[19] relies upon a series of CD analyses coupled with MD and DFT calculations as well as NMR experiments.[20,21] Collection of the CD spectra was obtained from MeOH solutions (10^{-5} M solutions) of diastereoisomers **14a,b** within a range of wavelength between 200–300 nm (Figure 2). The CD profile of compound **14a** has a positive absorption band at 212.9 nm and a second negative band centered at 226.5 nm. This profile can be addressed to a left β-

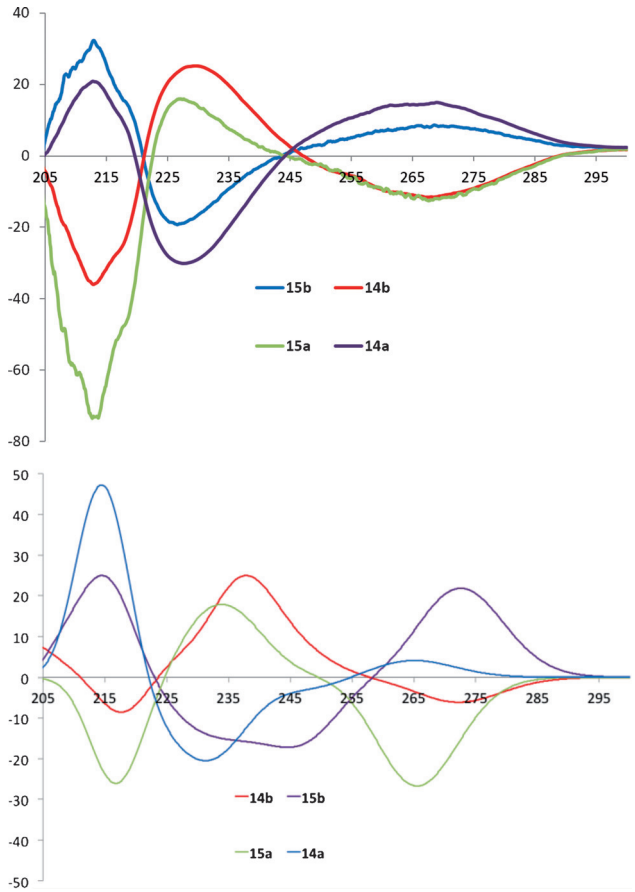


Figure 2. CD spectra of diastereoisomers **14a,b** and **15a,b** experimental (top), calculated (bottom)

turn in accordance with those reported in literature.[22] On the other side, the CD spectrum of **15a** shows a comparable trend with a negative absorption band at 213.0 nm and a second positive band centered at 227.6 nm, consistent for a right β-turn.

Similarly, the CD profile of compound **14b** has a negative absorption band at 212.9 nm and a second positive band centered at 229.6 nm. This profile can be addressed to a right β-turn in accordance with those reported in literature.[19]

On the other side, the CD spectrum of **15b** shows a positive absorption band at 212.8 nm and a second negative band centered at 227.7 nm, consistent for a left β-turn. Simulation of the CD absorption profile has been conducted by TD-DFT calculation at the B3LYP/6-31 g(d,p) level simulating a methanolic solution.[15]

The calculated CD spectra nicely fit with the experimental profiles indicating that inversion of the configurations at the isoxazoline-norbornane moieties correspond to an inversion of the chirooptical properties of the products at hand. As the calculated CD spectrum of the conformer **14a** nicely fit the experimental one obtained the configuration can be assigned beyond any reasonable doubt. The same approach was followed for all the other diastereoisomers (see the Supporting Information).

The extent of H-bonding in diastereoisomers **14,15** in $CDCl_3$ and DMSO was evaluated through temperature coefficients of the amide and carbamate protons at 298.15 and 318.15 K for $CDCl_3$ and up to 348.15 K for DMSO as well as DMSO-titration experiments.[22] It can be assumed that lower temperature coefficients values in $CDCl_3 \leq 2.4$ ppb/K are related not only to shielded protons but also to accessible ones; hence, only values significantly larger than 2.4 ppb/K in $CDCl_3$ can be unambiguously assigned to NH protons initially shielded, which become exposed to the solvent upon increasing temperature.[5,23]

On the other hand, low temperature coefficients in DMSO (< 5 ppb/K) are related to inaccessible protons to the solvent. Diluted solutions (10^{-3} M) of the compounds **14,15** in the deuterated solvents of choice were used to record the ^{1}H NMR spectra. Scheme 2 reports the temperature coefficients values expressed in Δppb/ΔK highlighted in red for $CDCl_3$ and in blue for DMSO. The results of the determinations indicate that in $CDCl_3$ the four diastereoisomers display somewhat a borderline situation since the values of the amide protons are in the range 2.5–5.0 Δppb/ΔK and similarly happens for the carbamate protons. The temperature coefficients in DMSO indicate that the amide protons, ranging at 3.2–4.2 Δppb/ΔK for all the four substrates, are inaccessible to the solvent, while carbamate protons have Δppb/ΔK values higher than the threshold values (8.6–9.2 Δppb/ΔK) indicating the accessibility of those protons to the solvent.

We also performed DMSO-titration experiments with gradual addition of DMSO to $CDCl_3$ solutions (10^{-3} M) of the four diastereoisomers **14,15**. Figure 3 shows the plots of the four compounds. The results clearly indicated that chemical shifts of the amide protons (solid lines with solid symbols) remain almost unchanged over the addition of increasing amounts of DMSO to the $CDCl_3$ solutions; the range of variation ΔNH is 0.09-0.18 ppm indicating the inaccessibility of the amide proton, engaged in H-bonding with the oxygen atom of the carbonyl group.

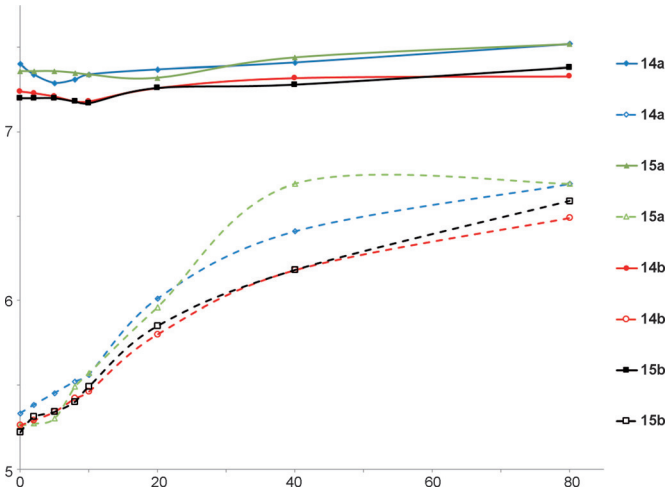


Figure 3. DMSO-titration of compounds **14,15**. Solid lines (solid symbols) indicate amide protons and dashed lines (open symbols) the carbamate protons.

A larger chemical shift variation is however observed for the carbamate NH proton (dashed lines with open symbols) with ΔNH values ranging from 1.23 to 1.43 ppm. The carbamate NH protons are prone to be attached by the DMSO molecule through a hydrogen bond, being more accessible. To properly rationalize all the data obtained from DMSO titration and VT-NMR experiments, and to better understand the dynamic behavior of **14,15**, unrestrained MD simulations were performed both in DMSO and water to compare theoretical calculations and NMR experiments as well as to probe their behavior in biological systems.[15]

Through the calculated trajectories it is possible to analyze the conformations assumed by the cyclopentane ring, which plays the pivotal role in the side chains pairing and then what kind of interactions occurs between these. In Figure 4, the dihedral angle values assumed by every compound along the simulation are reported in DMSO solutions (see the Supporting Information for simulation in water).

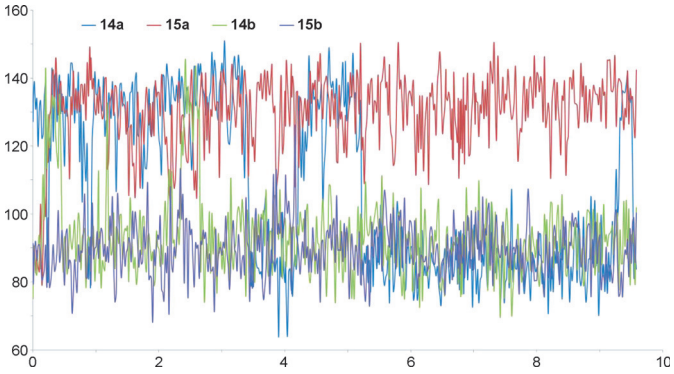


Figure 4. Dihedral angles ($H_{4isox.}$ and bridged methylene) values along 10 ns simulations in DMSO as solvent.

Dihedral angle values within 80° and 110° are consistent with boat-like conformations while higher values with chair-like conformations. Three out of four diastereoisomers spend more time with the cyclopentane methylene upward with the exception of **15b** showing a preference for a chair-like conformation. In the boat-like conformations all the geometrical requirements imposed by the definition of β-turn are respected (see the Supporting Information).

The reported results indicate that the chemistry of oxazanorbornenes offers the possibility to synthesized stereo-ordinated constrained aminols having the geometrical features to be used as turn inducers. These derivatives were spectroscopically characterized and their structures studied through NMR-titration and VT-NMR experiments. The measured temperature coefficients in DMSO are indicative for the existence of an intramolecular hydrogen bond that can occur when a boat-like conformation is present only. These findings are in agreement with the recorded CD spectra. Chirooptical properties revealed a β-turn arrangement of all the synthesized compounds, where, depending on the absolute configuration of the cyclopentane spacer, they can be labeled as left- or right-handed turns. Moreover, comparison between experimental and calculated CD spectra allowed for the identification of the absolute configuration of every single diastereoisomer.

The driving force that makes so efficient these compounds in inducing β-turns can be related to the presence of a fused isoxazolinic ring or similar rings to the cyclopentane moiety. This latter usually adopts an envelope conformation with the flap directed toward the isoxazoline ring thus giving a boat-like appearance to the bicyclic system. The chair-like conformation was found higher in energy (from B3LYP/6-31 g(d) optimized structures). The boat-like conformation allows for the relief of non-bonded interactions between the heterocyclic ring and the substituents on the adjacent cyclopentane carbons and causes the dihedral angles between the isoxazoline protons and the adjacent trans cyclopentane protons to be near 90°. This results in a flattening of the cyclopentane envelope pushing the amino and hydroxy groups close each other.[14b]

The reported diastereoisomeric compounds represents a "proof of concept" for the use of simple and easily prepared aminols for the synthesis of non-peptidic turn-inducers. It is worthwhile to note that the aminols themselves display the crucial role in the formation of the β-turn arrangement. Further experiments will aim to ascertain the influence of the nature of the two side chains on the type of turn for a better understanding of applicability/reliability of these scaffolds as β-turn inducers.

Experimental Section

Syntheses, characterization, and detailed procedures for all the compounds can be found in the Supporting Information.

Acknowledgements

Financial support: University of Pavia, MIUR (PRIN 2011, CUP: F11J12000210001) and COST Action CM1004.

Keywords: amino alcohols · β-turn inducers · cycloadditions · Diels–Alder reactions · nitrosocarbonyl compounds

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Received: August 4, 2015
Published online on September 29, 2015