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Peptidomimetics with tunable tertiary amide bond containing substituted β-proline and β-homoproline

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ABSTRACT

Tunable *cis*/*trans* prolyl amide bond configuration in substituted β-proline (β-Pro) and β-homoproline (β-Hpro) homodimers was explored, based on position and nature of the substituent. Tertiary amide bond in β-proline ($\beta^{2,3}$-substituted) dimers show distinct *trans* configuration and β-homoproline (β^{3}-substituted) dimers preferably exhibited *cis* configuration.

1. Introduction

Oligomers with well-defined conformations, known as foldamers,[1] mostly contain backbone intramolecular H-bonding network, which majorly contributes to the structural shape and stability in the foldamers.[2] Occasionally we come across certain class of oligomers, i.e., polyprolines[3–5] and peptoids,[6,7] which cannot have backbone H-bonding network as they lack backbone amide protons. Nevertheless, these classes of foldamers also form well-defined stable structures as noticed in PPII helices with poly prolines[4,5] and in peptoids with chiral centres at α-position of their *N*-substituents.[8]

In an ongoing study in our laboratory toward the development of short collagen related peptides (CRPs)[9] containing β-prolines and β-homoprolines that may spontaneously self assemble into the bioactive form of collagen to interact with collagen receptor sites with an aim to have new anti-thrombotic therapy, we were interested to understand the folding preferences of differently substituted β-Pro and β-Hpro containing short peptides. Controlling tertiary amide bond *cis*/*trans* isomerism is crucial in generating ordered structures in these classes of peptides, despite the fact that the energy difference between the two isomers is not significantly different. In nature, *cis*/*trans* isomerism of the prolyl–peptide bonds plays pivotal role in determining the structure, function of certain proteins and peptides.[10] Studies carried out in non-natural oligomers containing proline chimeras showed that the tertiary amide bond configuration correlates well with the nature and position of the substituent of the pyrrolidine ring.[11a–d] Fine tuning *cis*/*trans* isomerism in prolyl–peptide bonds remains a challenging task. While the *cis*/*trans* isomerization of α-proline derivatives and their control on structures have been studied extensively,[12] that of β-Hpro has been poorly explored. In 1998, Seebach et al. studied the conformational preferences of β-Hpro oligomers containing unsubstituted β^{3}-homoproline,[13] and crystal studies revealed that these oligomers formed conformations with exclusive *trans* amide bonds. Later Gellman et al., in 2003, conducted studies on β-peptides containing 2,2-disubstituted β^{2}-Hpro oligomers, and helical structures were demonstrated.[14] It was proposed that the disubstituted products will favour *cis* amide bonds preferably, thus favouring ordered structures. In PPII helices, both proline and hydroxyproline play predominant roles due to their predisposed backbone dihedral angles. However, to the best of our knowledge, there is no report, to date, related to any stable conformation formed from $\beta^{2,3}$- and β^{3}-substituted prolines. In an effort to understand the conformational preferences of substituted β-Pro and β-Hpro, we have carried out synthesis and solution conformational

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analysis on two varieties of β-prolines, i.e., $\beta^{2,3}$- (1) and β^{3}-substituted (2) monomers and their homodimers 3 and 4, respectively, supported by density functional theory (DFT)[15] calculations (Fig. 1).

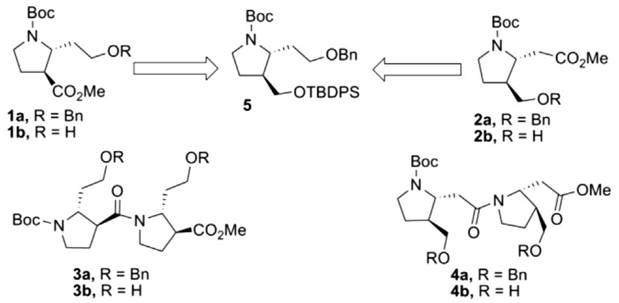
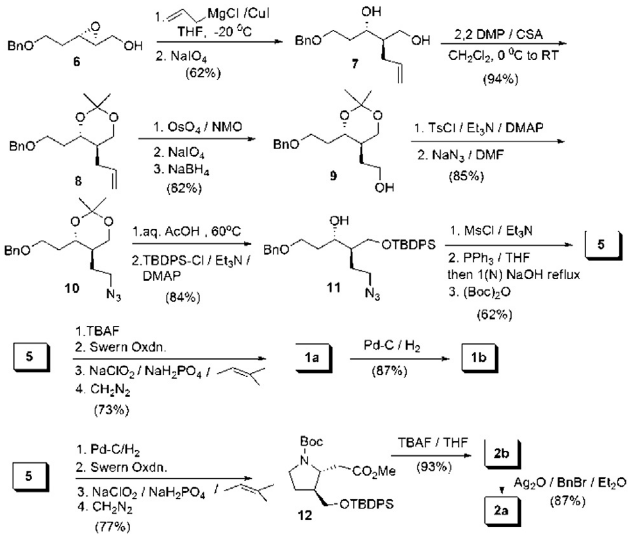


Fig. 1. Schematic representation of substituted β-Pro **1** and β-Hpro **2** and their homodimers, **3** and **4**, respectively.

2. Results and discussion

2.1. Synthesis of substituted $\beta^{2,3}$-proline 1 and β^{3}-homoproline 2

Monomers **1** and **2** were derived from a common precursor **5**, a 2,3-disubstituted pyrrolidine molecule having orthogonally protected groups that could be easily manipulated to prepare the desired β-prolines (Scheme 1). Syntheses of pyrrolidine precursor **5**, substituted β-proline **1** and β-homoproline **2** are depicted in Scheme 1. Treatment of the epoxide[16] **6** with 2.0 equiv of allylmagnesium chloride in THF at −80 °C produced 1,2-diol, exclusively. When this epoxide was reacted with Gilman reagent, prepared from 2.0 equiv of allylmagnesium bromide and 0.1 equiv of CuI, at −20 °C in THF, a mixture 1,2- and 1,3-diols was obtained. The crude reaction mixture was treated with $NaIO_4$ to remove the unwanted 1,2-diol and gave 1,3-diol **7** in 62% yield from epoxyalcohol **6**. The diol **7** was converted into acetonide **8** on treatment with 2,2-dimethoxypropane in CH_2Cl_2 in presence of catalytic amount of CSA in 94% yield. Dihydroxylation of the double bond on **8** with OsO_4 and oxidation of the resulting 1,2-diol with $NaIO_4$ furnished an aldehyde. Reduction of the aldehyde with $NaBH_4$ in MeOH gave the alcohol **9** in 82% yield from **8**. Compound **9** was converted into the azide **10** in 85% yield in two steps—tosylation with TsCl, Et_3N and catalytic amount of DMAP followed by reaction with NaN_3 in DMF at elevated temperature. The acetonide group on azide **10** was deprotected by treating it with an aqueous acetic acid solution at elevated temperature. Primary hydroxyl group of the diol, thus obtained, was selectively protected as its silyl-ether using TBDPS-Cl, Et_3N and catalytic amount of DMAP to produce azido-alcohol **11** in 84% yield from **10**. The azido-alcohol **11** on intramolecular cyclization provided the pyrrolidine **5** in 62% overall yield using the following steps—first treatment with MsCl and Et_3N in CH_2Cl_2 to give a mesylate, followed by reduction of the azido group with PPh_3 in THF with the resulting amine undergoing smooth intramolecular cyclization in situ on addition of 1(N) NaOH under reflux to produce the pyrrolidine backbone. Finally the amine group on the pyrrolidine ring was protected as Boc carbamate to provide **5**. Silyl-ether on **5** was cleaved with TBAF in THF to produce a primary alcohol. This alcohol was oxidized into an acid in two steps, Swern oxidation followed by oxidation of the resulting aldehyde using $NaClO_2$ along with $NaH_2PO_4 \cdot 2H_2O$ in a mixture of 2-methyl-2-butene and ^{t}BuOH (1:2) gave an acid. Acid was transformed into its ester **1a**, on treatment with excess of diazomethane in Et_2O, in 73% yield from **5**. Cleavage of benzyl ether of 1**a** with Pd–C in MeOH using a H_2-filled balloon produced **1b** in 87% yield.



Scheme 1. Syntheses of substituted $\beta^{2,3}$-proline **1** and β^{3}-homoproline **2**.

Synthesis of **2b** started with cleavage of benzyl ether on **5** with Pd–C under hydrogen in MeOH to provide a primary alcohol. Two step oxidation of the alcohol to an acid followed by esterification with diazomethane furnished the ester **12** in 77% yield from **5**. Silyl-ether on **12** was cleaved with TBAF in THF to produce the substituted β^{3}-Hpro **2b** in 93% yield. Free hydroxyl group of **2b** was further protected as benzyl ether with BnBr and Ag_2O in Et_2O to produce the side-chain benzylated β^{3}-Hpro **2a** in 87% yield.

Dimers **3a** and **4a** were obtained from **1a** and **2a**, respectively, following solution phase peptide coupling procedures using EDCI, HOBt and DIPEA. Hydrogenation of **3a** and **4a** with Pd–C in MeOH produced **3b** and **4b**, respectively.

2.2. Conformational studies

Conformational analyses of monomers (**1a**–**b** and **2a**–**b**) and dimers (**3a**–**b** and **4a**–**b**), with 10 mM concentration in $CDCl_3$, were performed using two-dimensional NMR techniques (Figs. 2 and 3) supported with DFT calculations. In **1a**–**b**, coupling constant value of $^{3}J_{C\alpha H-C\beta H}$ ~6.2 Hz was attributed to the dihedral angle '*θ*' [N–Cβ–Cα–C(O)] of about 120°.[17] The characteristic NOEs between CβH↔Cγ′$H_{(pro\text{-}R)}$, CαH↔Cγ′$H_{(pro\text{-}S)}$ support that the pyrrolidine ring takes $^{\beta}$E (Cβ-*endo*) conformation in both **1a**–**b** (Fig. 2A). Variation in the intensity of the CαH↔CδH NOE, strong in **1a** and weak in **1b**, suggests that χ^2 is varying to a value of 180° and 60° in **1a** and **1b**, respectively. DFT calculations were carried out, where potential energy surfaces for monomers, **1a**–**b** (tertiary butyl group at N-terminal replaced with CH_3) were explored at B3LYP[7c,18]/6-31G(d,p) level of theory in gas phase, to suggest that the *trans* conformation is the lowest energy conformations for both **1a**–**b**, shown in Tables S27–S29.[17]

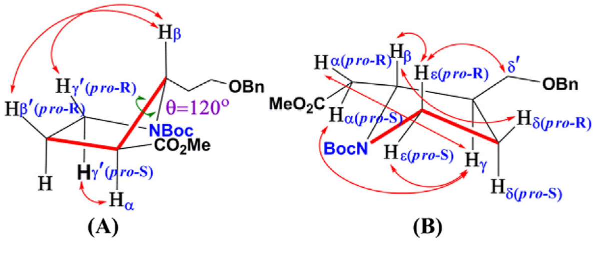


Fig. 2. Schematic representation of the proposed conformations with NOEs shown in arrows for (A) $^{\beta}$E (Cβ-*endo*) conformation of **1a**; (B) E_N (*N-exo*) conformation of **2a**.