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Locked Conformations for Proline Pyrrolidine Ring: Synthesis and Conformational Analysis of *cis*- and trans-4-*tert*-Butylprolines

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The motional restrictions of the proline pyrrolidine ring allow this secondary amine amino acid to act as a turn inducer in many peptides and proteins. The pyrrolidine ring is known to exhibit two predominant pucker modes (i.e., C-4 (C\(\uparrow\)) exo and endo envelope conformers whose ratio can be controlled by proper substituents in the ring). In nature, the exo puckered 4(R)-hydroxy-L-proline plays a crucial role as a building block in collagen and collagen-like structures. It has been previously concluded that the electronegativity of the 4-cis-substituent increases the endo puckering while the electronegativity of the 4-trans-substituent favors the exo puckering. Here, we have introduced a sterically demanding *tert*-butyl group at C-4 in trans- and cis-configurations. In the case of trans-substitution, the induced puckering effect on the pyrrolidine ring was studied with X-ray crystallography and \(^1\)H NMR spectral simulations. Both cis- and trans-4-*tert*-butyl groups strongly favor pseudoequatorial orientation, thereby causing opposite puckering effects for the pyrrolidine ring, cis-exo and trans-endo for L-prolines, in contrast to the effects observed in the case of electronegative C-4 substituents. The syntheses and structural analysis are presented for the conformationally constrained 4-*tert*-butylprolines. The prolines were synthesized from 4-hydroxy-L-proline, substitution with *t*-BuCuSPhLi being the key transformation. This reaction gave \(N\)-Boc-trans-4-*tert*-butyl-L-proline tert-butyl ester in 94% ee and 57% de. Enantioselectivity was increased to 99.2% ee by crystallization of \(N\)-Boc-trans-4-*tert*-butyl-L-proline in the final step of the synthesis.

Introduction and Background

Due to the conformational restrictions imposed by its pyrrolidine ring, the proteinogenic amino acid proline has an exceptional tendency to act as a turn inducer in peptides and proteins.\(^1\) The pyrrolidine ring exhibits two predominant pucker modes: C-4 (C\(\uparrow\)) exo and endo envelope conformers, that is, “up” and “down”, respectively (Figure 1).\(^2\) In the case of unsubstituted proline, the endo puckering mode is favored over the exo mode. The puckering propensity can be controlled by proper choice of ring substituents. In collagen structures, the...
Synthetic C-4 fluoroprolines have been used to elucidate the puckering effect of electronegative substituent where \textit{trans}-fluoroprolines promote \textit{exo} envelope and \textit{cis}-fluoroprolines promote the \textit{endo} envelope conformers. \textsuperscript{3,4} Extensive computational and NMR studies have suggested that the conformational effects of the electronegative substituent is dictated by inductive and stereoelectronic factors. Moreover, it has been concluded that the peptide cis/trans-isomerism in collagen triple helix structure is dictated by the stereochemistry (R/S) of the C-4 substituent (OH, F). \textsuperscript{5,6} It has also been suggested that the 4-hydroxyproline stabilized ring pucker is a key determinant endowing collagen its stability. \textsuperscript{7,8}

Although 4-substituted prolines have gained considerable synthetic interest, \textsuperscript{9-8} the conformational restrictions caused by this substitution have not been considered in the context of peptide secondary structures. As an exception, Koskinen et al. have synthesized conformationally constrained C-4 methyl prolins for peptidomimetics with the purpose of increasing conformational stability through only a minor chemical change on the natural amino acid. \textsuperscript{9,10} Secondary structure constraint was observed for the open chain 4-methylproline tetrapeptides (Ala-C(4)-MePro-Gly-Ala) in a conformational NMR study. \textsuperscript{10}

The sterically bulky tert-buty1 group is commonly used to lock a ring conformation, due to its tendency to orient equatorially for spatial and entropic reasons. For prolines, this approach has been applied at the C-3 and C-5 positions. Several synthetic routes toward C-4 alkylated proline have been explored, \textsuperscript{9,10} but to our knowledge no synthetic pathway has been reported for 4-tert-buty1 prolins. Yet, introduction of this bulky group specifically on carbon C-4 is attractive as it can be expected that the steric control takes place via pyrrolidine ring while the tert-buty1 substituents at both C-3 and C-5 positions sterically interfere with the backbone peptide bonds.

In addition to the C-4 tert-buty1 prolins, a number of 4-alkyl substituted prolines have already been synthesized \textsuperscript{9,10} and are widely used in pharmaceutical industry, such as angiotensin-converting enzyme (ACE) inhibitors and potential inhibitors of proline dehydrogenase. \textsuperscript{11} Our primary synthetic interest was the preparation of \textit{trans}-4-tert-buty1-L-proline. We present herein the synthetic access to all the C-4 tert-buty1 and C-2 CO\textsubscript{2}Bu epimers.

In this work, we also present a conformational study of the C-4 tert-buty1prolines based on X-ray diffraction data and \textsuperscript{1}H NMR spectroscopy.

**Results and Discussion**

Among the reported syntheses toward C-4 alkyl substituted prolines, the routes involving glutamate or pyroglutamate intermediates have proven to be successful in \textit{γ}-alkyllations. The alkyl groups have usually been introduced as electrophiles on glutamate enolates. \textsuperscript{9} However, the poor electrophilic nature of tert-buty1 group precludes this type of synthetic strategy for 4-tert-buty1prolines. Many alkylations routes involve synthetic steps via α-vinyl bonds and subsequent catalytic hydronation. \textsuperscript{12} Unfortunately, this type of synthetic route would involve unfavorable high energy intermediates caused by the allylic strain involving the tert-buty1 group. Alkyl cuprate substitutions of prolines at the C-4 position appealed to us as an intriguing approach.

Our synthesis began with the preparation of intermediate 4 starting from trans-4-hydroxyproline using literature procedures (Scheme 1). \textsuperscript{7,13} The hydroxyproline 4 was then submitted to a Mitsunobu-type bromination, yielding the bromo prolinate 5, key intermediate in this synthesis route. \textsuperscript{14} Bromide was substituted with a tert-buty1 group in a Corey–House reaction using tert-buty1licuprate as the nucleophile. \textsuperscript{15} The alklylation reaction was attempted with several types of tert-buty1 cuprates: Gilman (\textit{t}-Bu\textsubscript{2}CuLi), \textsuperscript{16-18} cyanato (\textit{t}-Bu\textsubscript{2}CuCNLi), \textsuperscript{19} and thiophenol (\textit{t}-Bu\textsubscript{2}CuSPhLi) \textsuperscript{17} reagents under various reaction conditions. \textsuperscript{16} We found out that the Posner tert-buty1 thiophenolcuprate procedure proved to be most efficient in the substitution. \textsuperscript{17} The substitution reaction proceeded as described for Scheme 1.
in THF at −18 °C in acceptable yield (54%) and gave 4-tert-butylprolinate 6 in 94% ee and 57% de. The yield was satisfactory, taking into account the reported modest yields for tert-alkylecuprates in general. This was especially the case, while our electrophile, inactivated secondary halogen in the 5-ring, is a poor candidate for substitution reactions.

The reason for the observed C-2 epimerization lies in the applied basic reaction conditions that led to enolate equilibrium and thus loss in diastereoselectivity. This was especially pronounced when the reaction times were prolonged.

In all cuprate reactions, some ring-opened N-Boc allyl glycine tert-butyl ester was found as a side product. This is most likely formed through direct transmetalation, and similar observations have been reported in the literature.8c

The diastereo- and enantioselectivities were determined by gas chromatography. Unfortunately, only the trans-enantiomers of tert-butylproline 6 of the four epimers were fully separable in chiral GC. These diastereomers were separable by flash chromatography. The absolute stereochemistries in prolinols 7 and 8 were determined through the corresponding Mosher esters. The enantiopurity of 8 was also determined through the Mosher ester. The final step in the synthesis involved TEMPO-catalyzed oxidation of the primary alcohols back to the corresponding substituted prolines 9 and 10.18 Although the oxidation proceeded in 80% (for 9) and 76% (for 10) yields and gave NMR pure samples, these products were recrystallized to improve enantiopurity, lowering the total yields to 57 and 48%, respectively. A small amount of proline 9 was esterified using isourea 3 to determine the enantiopurity with GC, and it was found to be excellent (99.2% ee).

An X-ray structure of proline 9 proved that the tert-butyl substituent is trans to the carboxyl group and occupies a pseudoaxial position in the crystal (Figure 2).

Determination of the enantiopurity also required the other enantiomers for accurate chiral GC analysis. Synthesis of the enantiomer for prolinate 6 was performed starting from the intermediate 11, which was prepared from trans-4-hydroxy-L-proline using literature procedures (Scheme 2).19 The stereochemistry at C-4 was inverted using a Mitsunobu reaction to give 12 in good yield (82%). The fully protected hydroxyproline 12 was hydrolyzed (1 M sodium hydroxide) to give N-Boc-trans-4-hydroxy-D-proline ent-2 in quantitative yield. Subsequent esterification with isourea 3 proceeded in 79% yield. The hydroxyprolinate ent-4 was brominated using Mitsunobu-type reaction with inversion at C-4. This key intermediate was then alkylated using the same conditions as for its enantiomer 5 using t-BuCuSPhLi. The reaction gave prolinate ent-6 in acceptable yield (34%) and in selectivity similar (92% ee, 68% de) to that of its enantiomer 6.

Pure trans-epimer of 6 was crystallized from freezer cold (−18 °C) isooctane, and the crystallographic structure was determined (Figure 3).

Solution structures were determined relying on vicinal \( \textit{J}_{\text{H-H}} \) coupling constants, which were obtained from spectral simulations. The simulated couplings were first converted into \( \text{H-C-C-H} \) torsion angles exploiting the Haasnoot–Altona equation (including the \( \beta \)-substituent correction) (Tables 1 and 2).

The reciprocal use of this formula yielded the estimated \( \textit{J}_{\text{H-H}} \) coupling constants from the corresponding structural dihedral parameters (Tables 1 and 2).

In the case of \textit{trans}-4-tert-butyl-L-proline, the calculated dihedrals were set as constraints in the geometry optimization (MM+ force field). As a result, unambiguous \textit{endo} puckering could be deduced for this proline. By comparing the simulated \( \textit{J}_{\text{H-H}} \) couplings at 25 and 110 °C, we found that there is still strong preference for \textit{endo} puckering mode even at elevated temperatures (Table 1).

The 4-tert-butyl-proline ring conformations were also studied computationally using B3LYP at 6-31G* level of theory. The structures were truncated from the peripheral tert-butyl ester and Boc-group for computational reasons (Figures 4 and 5). In the case of \textit{trans}-4-tert-butyl-L-proline geometry optimization, both \textit{exo} and \textit{endo} conformers were found as energy minima of which the \textit{endo} mode was energetically favored by 2.5 kcal/mol over its \textit{exo} counterpart. On the basis of the \( \textit{J}_{\text{H-H}} \) couplings

**TABLE 1.** Simulated and Calculated \( \textit{J}_{\text{H-H}} \) Couplings for \textit{trans}-4-tert-Butylproline

<table>
<thead>
<tr>
<th>coupled protons</th>
<th>NMR at 25 °C</th>
<th>NMR at 110 °C</th>
<th>( \textit{J}_{\text{H-H}} ) couplings (Hz) calculated from structural parameters</th>
<th>X-ray</th>
<th>DFT \text{endo} pucker</th>
<th>DFT \text{exo} pucker</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2^\text{H}_3^\text{a} )</td>
<td>9.26</td>
<td>9.13</td>
<td>9.55</td>
<td>8.16</td>
<td>8.52</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_2^\text{H}_3^\text{e} )</td>
<td>1.13</td>
<td>1.54</td>
<td>0.91</td>
<td>0.45</td>
<td>9.03</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_3^\text{a}-\text{H}_4 )</td>
<td>12.57</td>
<td>11.43</td>
<td>12.25</td>
<td>12.59</td>
<td>9.08</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_3^\text{e}-\text{H}_4 )</td>
<td>6.65</td>
<td>7.18</td>
<td>6.03</td>
<td>5.42</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_5^\text{a}-\text{H}_4 )</td>
<td>10.09</td>
<td>9.49</td>
<td>11.20</td>
<td>10.62</td>
<td>8.22</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_5^\text{e}-\text{H}_4 )</td>
<td>8.49</td>
<td>8.45</td>
<td>6.64</td>
<td>7.36</td>
<td>0.80</td>
<td></td>
</tr>
</tbody>
</table>

* Based on spectral iteration (see Experimental Section). * Calculated from torsion parameters using the Haasnoot–Altona equation (including the \( \beta \)-substituent correction).

**TABLE 2.** Simulated and Calculated \( \textit{J}_{\text{H-H}} \) Couplings for \textit{cis}-4-tert-Butylproline

<table>
<thead>
<tr>
<th>coupled protons</th>
<th>NMR at 25 °C</th>
<th>( \textit{J}_{\text{H-H}} ) couplings (Hz) calculated from structural parameters</th>
<th>X-ray</th>
<th>DFT \text{exo} pucker</th>
<th>DFT \text{endo} pucker</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{H}_2^\text{H}_3^\text{a} )</td>
<td>9.08</td>
<td>9.96</td>
<td>10.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_2^\text{H}_3^\text{e} )</td>
<td>7.99</td>
<td>7.96</td>
<td>6.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_3^\text{a}-\text{H}_4 )</td>
<td>12.17</td>
<td>12.61</td>
<td>11.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_3^\text{e}-\text{H}_4 )</td>
<td>6.98</td>
<td>5.40</td>
<td>4.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_5^\text{a}-\text{H}_4 )</td>
<td>11.24</td>
<td>11.25</td>
<td>10.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{H}_5^\text{e}-\text{H}_4 )</td>
<td>7.47</td>
<td>6.50</td>
<td>2.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on spectral iteration (see Experimental Section). * Calculated from torsion parameters using the Haasnoot–Altona equation (including the \( \beta \)-substituent correction).
It is also worth noticing that in the endo again indicates that pseudoequatorial orientation of the slightly below the plane. Inspection of simulated 3 nearly planar conformation, where the nitrogen is located the 2-carboxy group. This distorts the pyrrolidine to a puckering in the case of cis and exo puckering in the case of trans-

As a result of demonstrated conformational analysis, molecular modeling, and X-ray structural mimetics. Currently we are investigating structural effects of trans-4-tert-butylproline in β-turn mimetics.

**Experimental Section**

(2S)-N-Boc-trans-4-hydroxy-L-proline tert-Butyl Ester (4). A solution of proline 2 (3.96 g, 17.1 mmol, 100 mol %) in 60 mL of dry THF was treated with O-tert-butyldimethylsilanediisopropylsilane 3 (5.14 g, 25.7 mmol, 150 mol %) at room temperature and then stirred for 2.5 h at 60 °C. Additional O-tert-butyldiphenylsilane 3 (3.43 g, 17.1 mmol, 100 mol %) was added to the mixture, and then stirring was continued overnight. Precipitated urea was filtered off through Celite followed by ether washings, and the filtrate was evaporated in vacuo to give an oily white solid. The crude product was purified by flash chromatography (30–50% ethyl acetate/hexanes) to give the corresponding tert-butyl ester 4 as a colorless oil (3.93 g, 80%). Rf = 0.45 (ethyl acetate); [α]D = −57.3 (c 1.01, CHCl3); IR (thin film, cm⁻¹): 3437, 2978, 2934, 1742, 1703, 1403, 1367, 1151; 1H NMR (400 MHz, CDCl3): δ 4.43 (br s, 1H), [4.28 (t, 7.6 Hz), 4.24 (t, 7.6 Hz) 1H], [3.59 (d, 4.5 Hz), 3.56 (d, 4.3 Hz) 1H], [3.51 (d, 11.5 Hz), 3.39 (d, 11.1 Hz) 1H] [2.62 (s), 2.55 (s) 1H], 2.35–2.17 (m, 1H), 2.10–1.97 (m, 1H), 1.48–1.41 (m, 1H); 13C NMR (100 MHz, CDCl3): δ [172.1, 172.0], [154.4, 154.1], [81.1, 81.0], [80.1, 79.8], [70.0, 69.2], [58.5, 58.4], [54.6, 54.5], [38.1, 38.3], 28.3, [27.9, 27.8]; HRMS (ESI) calcd for C14H24NO4NaBr, 372.0786; found, [58.8, 58.7], [55.6, 55.3], [42.2, 41.5], [41.0, 40.0], [28.3, 28.2], 27.9; HRMS (ESI) calcd for C15H23NO6NaBr, 372.0786; found, 372.0777; Δ = 6.1 ppm. These data match those reported in the literature.7,24

N-Boc-cis-4-bromo-L-proline tert-Butyl Ester (5). Hydroxyproline 4 (4.61 g, 16.0 mmol, 100 mol %) and tetra-bromomethane (16.23 g, 48.9 mmol, 305 mol %) were dissolved in 40 mL of dry dichloromethane. The mixture was cooled to 0 °C, and triphenylphosphine (13.09 g, 49.9 mmol, 310 mol %) was added carefully. The reaction was stirred at room temperature for 15 h. Ethanol (4 mL) was added, and the solution was stirred for 2 h.Ether (40 mL) was added dropwise to precipitate the phenoxide oxide, which was filtered off, the filter cake was washed with dichloromethane (2 × 30 mL), and the filtrate was evaporated in vacuo to give a brown oil. Purification by flash chromatography (20–30% ether/hexanes) gave bromide 5 (4.17 g, 74%) as a colorless oil. Rf = 0.45 (50% ether/hexanes); [α]D = −38.1 (c 1.00, CHCl3); IR (thin film, cm⁻¹): 2978, 2932, 2924, 1746, 1704, 1395, 1367, 1151; 1H NMR (400 MHz, CDCl3): δ 4.43–4.15 (m, 2H), [4.05 (dd, 6.4 Hz, 11.8 Hz), 3.99 (dd, 6.4 Hz, 11.7 Hz) 1H], [3.69 (d, 5.8 Hz, 12.0 Hz), 3.65 (dd, 6.0 Hz, 11.9 Hz) 1H], 2.89–2.72 (m, 1H), 2.42–2.30 (m, 1H), [1.47 (s), 1.46 (s) 9H], [1.45 (s), 1.42 (s) 9H]; 13C NMR (100 MHz, CDCl3): δ [170.5, 170.3], [153.4, 153.2], [80.3, 80.2], [58.8, 58.7], [55.6, 55.3], [42.2, 41.5], [41.0, 40.0], [28.3, 28.2], 27.9; HRMS (ESI) calcd for C14H24NO4BrO2, 354.0635; found, 354.0581; Δ = 2.4 ppm. This compound is important for the synthesis of strained peptide turns. Currently we are investigating structural effects of trans-4-tert-butylproline in β-turn mimetics.

**Conclusions**

We have developed selective synthetic routes to both trans- and cis-4-tert-butylprolines from trans-4-hydroxy-proline using t-BuCuSPPhLi mediated substitution via a secondary bromide. According to 1H NMR-based conformational analysis, molecular modeling, and X-ray structure, the C4 tert-butyl group prefers strongly the pseudoequatorial orientation in the pyrrolidine ring and thereby promotes conformational ring locking for exo puckering in the case of cis and endo puckering in the case of trans-substituted prolines, in both solution and solid state. As a result of demonstrated conformational locking effect, the C4 tert-butyl-substituted prolines offer potentially very attractive tools to construct short con-

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Evaporation of solvent in vacuo gave a yellow oily solid. Silica gel chromatography (5–10% ether/hexanes) yielded prolinate 6 (0.469 g, 1.51 mmol, 100% ee, 57% de) as a white solid. Rf = 0.54 (50% ether in hexanes).

trans-1-6: IR (thin film, cm⁻¹): 2965, 1742, 1704, 1394, 1366, 1176, 1153, 1242; 'H NMR (400 MHz, CDCl₃); δ [4.24 (dd, 7.8 Hz, 4.15 (d, 8.6 Hz) 1H), [3.58 (dd, 8.6 Hz, 10.2 Hz), 3.48 (dd, 8.7 Hz, 10.6 Hz) 1H], [3.10 (t, 10.2 Hz), 3.03 (t, 10.2 Hz) 1H], 2.23–2.06 (m, 1H), 2.20–1.80 (m, 2H), [1.46 (s), 1.44 (s), 1.42 (s) 1H], 0.87 (s, 9H); 13C NMR (100 MHz, CDCl₃); δ [172.3, 172.2, 154.4, 154.0], [80.8, 80.8], [79.5, 79.4], [60.1, 59.9], [47.5, 47.3], [47.5, 46.6], 31.7, [30.9, 30.9], [28.4, 28.3], [28.2, 27.3]; HRMS (ESI): calculated for C₁₆H₂₃NO₃Na, 290.1889; found, 290.1886. 

cis-1-6: 'H NMR (400 MHz, CDCl₃); δ [4.11 (dd, 7.3 Hz, 8.6 Hz), 4.07 (dd, 8.2 Hz, 8.8 Hz) 1H], [3.66 (dd, 7.8 Hz, 9.8 Hz), 3.49 (dd, 7.5 Hz, 10.0 Hz) 1H], [3.10 (t, 11.0 Hz), 3.08 (t, 10.6 Hz) 1H), 2.29–2.18 (m, 1H), 2.06–1.91 (m, 1H), 1.65–1.55 (m, 1H), [1.46 (s), 1.45 (s), 1.45 (s), 1.43 (s) 1H], [0.90 (s), 0.89 (s) 9H]; 13C NMR (100 MHz, CDCl₃); δ 172.4, 154.0, 80.7, 79.7, [60.0, 60.0], [49.0, 48.2], [47.9, 47.8], 32.2, [31.1, 30.9], [28.4, 28.3], [28.0, 27.9], [27.5, 27.4]; GC Rf = 94.5 min.
Δ = 0.4 ppm. These data are consistent to those reported in the literature for the enantiomer 2.25

Acknowledgment. Financial support from the National Technology Agency of Finland, the Academy of Finland, and EU COST action D28 is gratefully acknowledged.

Supporting Information Available: Table of the optimization conditions for the tert-butyl copper alkylation. Experimental procedures for ent-4, ent-5, and ent-6. Copies of 1H and 13C NMR spectra of compounds 5–10, 12. GC chromatograms for compounds 6, ent-6, and tert-butyl ester derivative 9. Mosher ester analysis for compound 7 and 8. Crystallographic collection data and tables for 6 and 9. This material is available free of charge via the Internet at http://pubs.acs.org.

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