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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 (Cç) 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 1H 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 tert-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 (Cç) 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 nonproteinogenic amino acid, 4-(R)-hydroxy-L-proline (trans-substituted), is equipped with a C-4 (R) OH group that makes the exo puckering prevailing (Y = OH, X = H, Figure 1).3

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[FIGURE 1. Proline ring puckering modes are generally referred to C-4 (Cç) “endo” (or “down”) and “exo” (or “up”). Notations arise from the N1 – C4 pseudorotation angles; endo is associated to the gauche+ and exo to the gauche- torsion rotamer. The substitution of C-4 affects not only the endo and exo puckering but also the trans and cis peptide bonding.]

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Synthetic C-4 fluoroprolines have been used to elucidate the puckering effect of electronegative substituent where trans-fluoroprolines promote exo envelope and cis-fluoroprolines promote the endo envelope conformers. 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). 5 It has also been suggested that the 4-hydroxyproline stabilized ring pucker is a key determinant endowing collagen its stability. 6

Although 4-substituted prolines have gained considerable synthetic interest, 7–8 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 prolines for peptidomimetics with the purpose of increasing conformational stability through sterically interfering with the backbone peptide bonds. 9

In addition to the C-4 tert-butyl prolines, a number of 4-alkyl substituted prolines have already been synthesized 10–12 and are widely used in pharmaceutical industry, such as angiotensin-converting enzyme (ACE) inhibitors and potential inhibitors of proline dehydrogenase. 13 Our primary synthetic interest was the preparation of trans-4-tert-butyl-L-proline. We present herein the synthetic access to all the C-4 tert-butyl and C-2 CO₂Et-Bu epimers. In this work, we also present a conformational study of the C-4 tert-butylprolines based on X-ray diffraction data and 1H 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 γ-alkylations. The alkyl groups have usually been introduced as electrophiles on glutamate enolates. 6 However, the poor electrophilic nature of tert-butyl group precludes this type of synthetic strategy for 4-tert-butylprolines. Many alkylation steps via α-vinyl bonds and subsequent catalytic hydrogenation. Unfortunately, this type of synthetic route would involve unfavorable high energy intermediates caused by the allylic strain involving the tert-butyl group. Alkyl cuprate substitutions of prolines at the C-4 position appealed to us as an intriguing approach. 7

Our synthesis began with the preparation of intermediate 4 starting from trans-4-hydroxyproline using literature procedures (Scheme 1).7,8 The hydroxyproline 4 was then submitted to a Mitsunobu-type bromination, yielding the bromo proline 5, key intermediate in this synthetic route. 14 Bromide was substituted with a tert-butyl group in a Corey–House reaction using tert-butylcucurate as the nucophile. 15 The alkylation reaction was attempted with several types of tert-butyl cuprates: Gilman (t-Bu₂CuLi), 8,14,15 cyan (t-Bu₂CuCNLi₂), 14 and thiophenol (t-Bu₂CuSPhLi) 17 reagents under various reaction conditions. 16 We found out that the Posner tert-butyl thiophenolcuprate procedure proved to be most efficient in the substitution. 17 The substitution reaction proceeded

References


(6) See Supporting Information for optimization of the reaction conditions.
in THF at \(-18^\circ C\) 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-alkylcuprates 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 6 were fully separable in chiral GC. 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 pseudoequatorial 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 (\(-18^\circ C\)) isooctane, and the crystallographic structure was determined (Figure 3).

\[ \text{SCHEME 1. Synthesis of N-Boc-Protected 4-tert-Butylprolines} \]

\[ \text{FIGURE 2. ORTEP plot of the X-ray crystal structure of proline 9.} \]
Solution structures were determined relying on vicinal $(3J_{\text{H-H}})$ 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 $\gamma$-substituent correction) (Tables 1 and 2).20,21 The reciprocal use of this formula yielded the estimated $(3J_{\text{H-H}})$ coupling constants from the corresponding structural dihedral parameters (Tables 1 and 2).

In the case of trans-4-tert-butyl-L-proline, the calculated dihedrals were set as constraints in the geometry optimization (MM+ force field). As a result, unambiguous endo puckering could be deduced for this proline. By comparing the simulated $(3J_{\text{H-H}})$ couplings at 25 and 110 °C, we found that there is still strong preference for 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.22 For density functional theory (DFT) modeling, the structures were truncated from the peripheral tert-butyl ester and Boc-group for computational reasons (Figures 4 and 5). In the case of trans-4-tert-butyl-L-proline geometry optimization, both exo and endo conformers were found as energy minima of which the endo mode was energetically favored by 2.5 kcal/mol over its exo counterpart. On the basis of the $(3J_{\text{H-H}})$ couplings optim...
deduced from the X-ray structure and DFT models, it is evident that trans-4-tert-butylproline exists predominantly in the endo puckering conformation.

The DFT modeling of the cis-4-tert-butyl-D-proline again indicates that pseudoequatorial orientation of the tert-butyl group is clearly preferred: the exo conformer is 3.3 kcal/mol lower in energy than the endo one (Figure 5). It is also worth noticing that in the endo conformer the 4-tert-butyl group is spatially in a close proximity to the 2-carboxy group. This distorts the pyrrolidine to a slightly below the plane. Inspection of simulated J_H-H couplings and the calculated ones (Table 2) shows apparent preference for exo puckering mode for the cis-tert-butyl proline, although this was less pronounced than the endo puckering in the case of the trans-substitution.

Conclusions

We have developed selective synthetic routes to both trans- and cis-4-tert-butylprolines from trans-4-hydroxyproline using t-BuCuSPhLi mediated substitution via a secondary bromide. According to 1H NMR-based conformational analysis, molecular modeling, and X-ray structure, the C-4 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 C-4 tert-butyl-substituted prolines offer potentially very attractive tools to construct short constrained peptide turns. Currently we are investigating structural effects of trans-4-tert-butylproline in β-turn mimetics.

Experimental Section

(25)-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 tert-butyl N,N-diisopropylprolisourea 3 (5.14 g, 25.7 mmol, 150 mol%) at room temperature and then stirred for 2.5 h at 60 °C. Additional tert-butyl N,N-diisopropylprolisourea 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%).

(25)-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 phosphine 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. ∆ _R = 0.45 (50% ethyl acetate/hexanes); [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), 1.49–1.41 (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, 38.2], 28.3, [27.9, 27.8]; HRMS (ESI) calcd for C₁₄H₂₅NO₅Na, 310.1630; found, 310.1649; ∆ = 6.1 ppm. These data match those reported in the literature.²³,²⁴

(25)-N-Boc-cis-4-bromo-L-proline tert-Butyl Ester (5). A solution of proline 2 (3.96 g, 17.1 mmol, 100 mol%) in 60 mL of dry dichloromethane was stirred for 20 min, and a precooled (-77 °C) solution of CuI (1.47 g, 8.52 mmol, 600 mol%) and tetra-diisopropylisourea in pentane (4.6 mL, 8.52 mmol, 600 mol %) was added. The reaction was stirred at -18 °C for 16 h and treated with careful addition of 1.85 M diisopropylisourea to give a colorless oil (3.93 g, 80%).


Evaporation of solvent in vacuo gave a yellow oily solid. Silica gel chromatography (5–10% ether/hexanes) yielded prolinate (6) (0.153 g, 54% ee, 57% de) as an oily white solid. Rf = 0.54 (50% ether in hexanes).

**trans-1-6**: IR (thin film, cm⁻¹): 2965, 1742, 1704, 1394, 1366, 1176, 1153, 1214; 'H NMR (400 MHz, CDCl₃): δ [4.24 (d, 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) 18H], 0.87 (s, 9H); 13C NMR (100 MHz, CDCl₃): δ [172.8, 172.2], [154.4, 154.0], [80.6, 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 C₁₄H₂₇NO₃Na, 280.1889; found, 280.2.352; Δ = 1.4 ppm; gc T₆ = 79.4 min.

**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) 18H], [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 T₆ = 94.5 min.  

**Recrystallization**: Recrystallization from MeOH/CH₂Cl₂ (1 mL)/hexanes (10 mL) gave white crystals (0.160 g, 57%).

**trans-1-7**: To a solution of prolinate (0.263 g, 1.02 mmol, 100 mol %) in acetonitrile (0.5 mL) and distilled water (0.5 mL) was stirred for 21 h at room temperature. The reaction was quenched by addition of saturated aqueous Na₂SO₄ solution until color had disappeared. Aqueous phase was evaporated under reduced pressure, and the acetic acid mixture was basified (pH ≈ 10) with 1 M NaOH solution, washed twice with ether, and acidified with 1 M HCl solution (pH ≈ 3). The acetic acid phase was extracted three times with ether. The combined organic extracts were filtered and 2.0 ppm.  

**trans-1-10**: A suspension of prolinate (8 28 mg, 0.108 mmol, 100 mol %) in acetonitrile (0.5 mL) and distilled water (0.5 mL) was evaporated under reduced pressure, and the aqueous mixture was basified (pH ≈ 10) with 1 M NaOH solution, washed twice with ether, and acidified with 1 M HCl solution (pH ≈ 3). The acetic acid phase was extracted three times with ether. The combined organic extracts were filtered and 2.0 ppm.  

**trans-1-12**: To a solution of prolinate (11) (0.50 g, 1.93 mmol, 100 mol %), triphenyl phosphine (1.01 g, 3.86 mmol, 200 mol %), and glacial acetic acid (0.22 mL, 3.86 mmol, 200 mol %) in dry THF (5 mL) was added 40% DEAD in toluene (1.67 g, 17.6 mL, 8.86 mmol, 200 mol %) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred overnight. The solvent was evaporated, and the residue was purified by silica gel chromatography (20–40% ether/hexanes) to give prolinate (12) (0.482 g, 82%) as a yellow oil. Rf = 0.24 (50% ether in hexanes); [α]D = +137.4 (c 1.00, CHCl₃); IR (thin film, cm⁻¹): 3411, 2961, 2872, 1731, 1637, 1437, 1405, 1365, 1252, 1167, 774; 1'H NMR (400 MHz, CDCl₃): δ [4.30 (t, 8.0 Hz), 4.20 (t, 7.8 Hz) 1H], 3.73–3.54 (m, 1H), [3.15 (t, 10.6 Hz), 3.03 (t, 10.2 Hz) 1H], 2.55–2.15 (m, 1H), 2.10–1.65 (m, 2H), [1.48 (s), 1.43 (s) 9H], 0.91 (s, 9H); 13C NMR (100 MHz, CDCl₃): δ [178.7, 175.0], [156.5, 153.7], [81.5, 80.4], [59.6, 59.4], 48.5, [48.3, 47.7], [32.3, 30.3], 30.8, [28.3, 28.2], 27.5; HRMS (ESI) calculated for C₁₈H₃₅NO₄Na, 324.1423; found, 324.1389; Δ = 7.7 ppm.

**trans-1-13**: A solution of prolinate (12) (0.424 g, 1.40 mmol) in THF (5 mL) was treated with aqueous 15 wt % NaOH solution and was stirred for 3 h. Most of the THF was evaporated under reduced pressure, and the aqueous mixture was acidified with 6 M HCl to pH ≈ 3. The mixture was extracted with ethyl acetate (10 times) until TLC showed no product in the aqueous phase. The combined organic extracts were dried over Na₂SO₄, filtered, and evaporated in vacuo to give prolinate (12) (0.385 g, 100 %) as a white solid. Rf = 0.50 (50% methanol in ethyl acetate); IR (thin film, cm⁻¹): 3500–2300 (broad band), 3419, 2979, 2935, 1725, 1671, 1421, 1197; 'H NMR (400 MHz, CDCl₃): δ 4.55–4.35 (m, 2H), 3.70–3.45 (m, 2H), 2.59–2.05 (m, 2H), [1.47 (s), 1.42 (s) 9H]; 13C NMR (100 MHz, CDCl₃): δ [128.7, 174.5], [156.7, 153.9], [81.9, 80.9], [69.6, 68.4], 57.8, 56.6, [59.0, 57.2]; HRMS (ESI) calculated for C₁₄H₂₀NO₄, 254.1005; found, 254.1005.
$\Delta = 0.4$ ppm. These data are consistent to those reported in the literature for the enantiomer 2.25

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**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 $^1$H and $^{13}$C NMR spectra of compounds 5–10, 12. GG 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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