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Synthesis of Fmoc-Protected trans-4-Methylproline

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Fmoc-protected trans-4-methylproline was synthesized starting from d-serine. The chiral scaffold of serine in the form of olefinated Garner’s aldehyde 3 was used to control the diastereoselective formation of the new stereocenter on the hydrogenation of allylic alcohol 4. The diastereoselectivity (syn/anti ratio) of the process was 86:14, attained with Raney nickel. Hydrogen migration seems not to be the sole factor lowering the diastereoselectivity, as nickel is known not to promote double-bond migration. Instead, the moderate stereoselectivity is attributed to the mobility of the side chain of 4, which allows the attack of hydrogen on both faces of the olefin (open transition state). A series of transformations led to ring precursor 8, which after recrystallization afforded the syn diastereoisomer in dr = 95:5. Protected trans-4-methylproline 11 was obtained from 8 in a straightforward fashion.

Introduction and Background

Substituted prolines are interesting targets as they are considered to be constrained analogues of natural amino acids.1 Thus, their incorporation into bioactive peptides renders conformationally constrained peptides. Such so-called peptidomimetics (or peptide mimics) are useful tools for the development of superior pharmaceutical agents and for establishing structure–bioactivity relationships, which could aid in the understanding of biological processes.1

While efficient asymmetric syntheses of 3- and 5-substituted prolines have been achieved,2 only a few approaches have been reported for 4-substituted prolines.1 Thus, in 1989, Koskinen and Rapoport3b described the synthesis of various cis- and trans-4-alkyl- and -phenylprolines by conversion of 4-substituted glutamic acid esters to the corresponding 5-hydroxypentanoic acids, followed by separation of diastereoisomers and intramolecular nitrogen alkylation. trans-4-Phenylproline has also been synthesized from 4-hydroxyproline3b by tosyl formation and further substitution of that group with lithium diphenylcuprate.4c,d Although the reaction proceeds with retention of configuration at the carbon bearing the tosylxy group, participation of the N-Boc protecting group results in the epimerization of the α-carbon, leading to 2:1 or 2:3 mixtures of diastereomers. Better results in terms of diastereomeric ratios were attained via Friedel–Craft reaction of benzene with different trans-4-hydroxyproline derivatives (pure cis- and trans-4-phenylproline were obtained)5b and from 4-ketoproline (cis-4-phenylproline was obtained pure and the trans diastereomer as a 9:1 mixture).1b Thottathil and co-workers1b reported the synthesis of trans-4-cyclohexylproline from pyroglutamic acid, in good yield and complete diastereoselectivity. Recently, Sasaki and co-workers1h described the synthesis of a proline structure with two discernible hydroxy functionalities, which according to the authors, could provide a range of 4-substituted proline derivatives.

Proline is known to have profound conformational effects in the tertiary structures of peptides and proteins.3 In connection with our studies on the structure and biosynthesis of collagen,4 we needed an efficient synthesis of trans-4-methylproline,5 suitable for incorporation in automated peptide synthesis. The free amino acid was isolated from apples by Hulme and Arthington in 1954,6,ab and its first synthesis was reported in 1962 by Gray and Fowden.5c Those authors hydrogenated the naturally occurring 4-methyleneproline with Adam’s catalyst, obtaining cis-4-methylproline as the major diastereoisomer. Later on, Lavergne and co-workersd obtained a 1:1

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mixture of cis- and trans-4-methylproline by cyclization of \( \delta \)-chlorinated compounds obtained by irradiating N-chloro-L-α-amino acids. In 1988, Belokon and co-workers\(^8\) described the synthesis of a 2:1 cis/trans-4-methylproline mixture, via condensation of glycine with activated olefins.

In view of the mentioned results, we were challenged to develop a new synthetic strategy for trans-4-methylproline. In this paper, we describe the synthesis of Fmoc-protected trans-4-methylproline. We believe that this route also constitutes a general synthetic pathway to 4-substituted prolines.

On the basis of retrosynthetic analysis, the 4-substituted proline structure can be reached from serine-derived \( \alpha \)-enoates, as outlined in Scheme 1.

Some of our preliminary results on the hydrogenation of \( E \)-enoates derived from fully protected serinal\(^6\) prompted us to further studies on the selectivity of the process, and to develop a synthetic route to 4-alkylprolines.

Results and Discussion

The synthetic pathway proposed for Fmoc-protected trans-4-methylproline is based on a diastereoselective approach, starting from the Garner's aldehyde (1, Scheme 2).\(^7\)

A Wittig reaction between phosphorane 2 and the Garner's aldehyde (1) provided \( \alpha _x \beta _\text{ unsaturated ester} 3\) in 72% yield, with the \( E/Z \) ratio \( \geq 96:4 \) (the \( Z \)-enoate was not observed by \( ^1 \text{H} \) NMR), as we have already described.\(^9\)

The enantiomeric excess of 3 was determined following the method described by Mann and co-workers.\(^8\) Thus, the hemiaminal moiety of 3 was hydrolyzed (p-TsOH in MeOH, rt, 2 h), and the resulting alcohol group was treated with Mosher's reagent.\(^9\) Analysis of the resulting Mosher ester by HPLC (Chiralcel OD column, Hex/IPA 95:5, 1 mL/min, 254 nm; \( t_{\text{R}(\text{S})} = 16.8 \text{ min}, \ t_{\text{R}(\text{R})} = 19.0 \text{ min} \) ) and \( ^1 \text{H} \) NMR gave 96% ee for 3, revealing that minimal racemization had occurred. This result is in agreement with that of Mann and co-workers,\(^8\) who reported 98% ee for a compound similar to 3 (containing a hydrogen atom instead of the 2-methyl group).

Previous studies carried out in our laboratory on the reduction of the double bond of 3 using different heterogeneous catalysts gave as the best result a 5:1 \( \text{syn/anti diastereomeric ratio} \) for the corresponding saturated ester (using \( \text{Pt/C} \) in Hex).\(^6\) The moderate diastereoselectivity was rationalized on the basis of a hydrogen migration, which results in the isomerization of the double bond.\(^4\)

With the aim to improve those results, we next attempted the hydrogenation of allylic alcohol 4, obtained in 88% yield by reduction of the ester functionality of 3 with \( \text{DIBAL-H} \) in toluene. We hoped that the hydroxyl moiety could interact strongly with the catalyst, providing a more rigid transition state that could favor the diastereofacial discrimination. Initially, hydrogenation of 4 was carried out on 10% \( \text{Pt/C} \). Slightly surprisingly, the deoxygenated compound (5a) was formed as the major product of the hydrogenation reaction (80% yield), even in hexane. In light of the literature,\(^6\) the acidic character of charcoal seems to be responsible for the large extent of the deoxygenation process. To avoid the cleave of the allylic C–O bond, neutral catalysts in basic media were tested. The results obtained are summarized in Table 1.

The diastereomeric purity of 5a was not detected in any experiment, which confirmed that its formation is an acid-catalyzed process. Clean formation of saturated alcohol 5b occurred with moderate selectivity. Palladium was discarded as it is the most active deoxygenating catalyst.\(^10\) Platinum oxide was used instead. Although it is known that platinum promotes less hydrogen migration than palladium,\(^10\) only a 75:25 \( \text{syn/anti ratio} \) was afforded with platinum oxide (Table 1, entry 1). The use of iridium was attempted, as according to Rylander,\(^11\) iridium is the metal with the greatest ability to deliver hydrogen in a cis manner, which is essential to attain selectivity. Iridium on charcoal in ethyl acetate turned out to be inactive even to promote deoxygenylation of allylic alcohol 4. In view of those results, we decided to turn to Raney Nickel, catalyst that does not promote isomerization (Table 1, entries 4–11).\(^10\) As the diastereoselectivity increased only to a maximum of 86:14 \( \text{syn/anti ratio} \), other factors in addition to double bond migration are envisaged to work against the diastereoselectivity of the process. Namely, the conformational mobility of allylic alcohol 4 must allow the compexion of the metal on the two faces of the double bond, lowering the diastereofacial selectivity.

Replacement of the hydroxy functionality of compound 5b (86:14 \( \text{syn/anti ratio} \)) by chloride (\( \text{PPh}_3, \ K_2\text{CO}_3, \ CCl_4 \) in \( \text{MeCN} \))\(^12\) had as a drawback the difficult separation of the phosphorus-containing compounds from the target molecule, which resulted in a lower yield of chloride 6a. Moreover, cleavage of the hemiaminal protecting group leading to 7a occurred to a large extent during flash chromatography, even using pretreated moisture-free silica gel. On the other hand, exchange of the alcohol group by bromide was a much easier reaction to work up and rendered compound 6b in high yield (90%). Hydrolysis of the hemiaminal moiety of 6b was achieved with 80% \( \text{AcOH} \) at 50 °C (91%).\(^13\) Benzylation of the alcohol group of 7a and 7b (\( \text{PhCOCl}, \ \text{Py}, 95% \) ) rendered ring precursors 8a and 8b, respectively, as white solids. Recrystallization of 8a (dr = 86:14) from hexane/ether 4:1 provided diasteromer (2R,4S)-8 in dr = 95:5. The diastereomeric purity of compound 8b was also increased by recrystallization (the conditions were not optimized).

Cyclization of (2R,4S)-8 (dr = 95:5) was carried out in tetrahydrofuran, using \( \text{KHMDS} \) as the base. The yield

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of the cyclization process is not affected by the temperature or the nature of the halogen (for 8a: 70% yield after 15 min at 40 °C and 72% yield at 0 °C; for 8b: 69% yield after 15 min at 0 °C). This fact can be expected taking into account the fast reaction rate. The reaction yield decreases as a result of the competing basic hydrolysis (aminolysis) of the Boc and benzoyl groups promoted by the base (KHMDS). The resulting amino alcohol is very soluble in water and is lost during work up. This problem is overcome when protected prolinol 9 is not isolated (vide infra).

Various attempts to deprotect the benzoyl group of 9 under basic conditions (NaOH) led to mixtures of compounds in which cleavage of the Boc group was observed. The problem was solved by hydrolyzing both the Boc and the benzoyl groups under acidic conditions (1 N HCl, 100 °C). The resulting amino alcohol is very soluble in water and is lost during work up. This problem is overcome when protected prolinol 9 is not isolated (vide infra).

Conclusions

We have developed a new direct route to trans-4-alkylprolines, using a substrate directed hydrogenation reaction for the synthesis of alcohol 5b. The route involves eight steps, renders only the trans diastereoisomer (after recrystallization), and should be applicable to other targets of the same family. We are currently investigating new approaches to improve the diastereoselectivity of the hydrogenation step. Moreover, the target trans-4-methylproline is being incorporated into small peptides in order to obtain backbone and side chain conformationally constrained peptides to mimic β-turns in proteins.

Experimental Section

General Methods. 1H NMR spectra were recorded on a Varian Unity-400 at 400 MHz and are reported in ppm downfield from SiMe 4. J values are given in Hz. 13C NMR spectra were recorded on a Varian Unity-400 at 100 MHz. HRMS were conducted on JEOL JMS-DX303 (EI+) and Micromass LCT (ES+) spectrometers. Optical rotations were measured on a Perkin-Elmer 343 polarimeter at room temperature, using a cell of 1 dm of length and ι 598 nm. Data are reported as follows: [a]D (solvent, concentration in g/100 mL). HPLC analyses were performed using a Waters system with UV detector, on a Chiralpak AS (Daicel) column. Melting points (mp) were performed on a Gallenkamp apparatus, using open capillary tubes. Values are given in degrees Celsius (uncorrected). Analytical thin-layer chromatography (TLC) was performed on Merck silica gel plates with QF-254 indicator.

Table 1. Hydrogenation of Allylic Alcohol 4

<table>
<thead>
<tr>
<th>entry</th>
<th>metal catalyst (substrate/catalyst)</th>
<th>solvent</th>
<th>additive</th>
<th>syn/anti ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PtO (4:1)</td>
<td>EtOH</td>
<td>NaNO2</td>
<td>75:25</td>
</tr>
<tr>
<td>2</td>
<td>PtO (4:1)</td>
<td>Hex</td>
<td>Et3N</td>
<td>70:30</td>
</tr>
<tr>
<td>3</td>
<td>PtO (8:1)</td>
<td>Hex</td>
<td>Et3N</td>
<td>70:30</td>
</tr>
<tr>
<td>4</td>
<td>Raney Ni (1.5:1)</td>
<td>EtOH</td>
<td></td>
<td>80:20</td>
</tr>
<tr>
<td>5</td>
<td>Raney Ni (2.7:1)</td>
<td>EtOH/AcOEt</td>
<td>70:30</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Raney Ni (1.5:1)</td>
<td>MeOH</td>
<td></td>
<td>85:15</td>
</tr>
<tr>
<td>7</td>
<td>Raney Ni (1.5:1)</td>
<td>MeOH/H2O</td>
<td>75:25</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Raney Ni (8:1)</td>
<td>MeOH</td>
<td></td>
<td>85:15</td>
</tr>
<tr>
<td>9</td>
<td>Raney Ni (20:1)</td>
<td>MeOH</td>
<td>80:20</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Raney Ni (2:1)</td>
<td>MeOH</td>
<td>80:20</td>
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<tr>
<td>11</td>
<td>Raney Ni (2:1)</td>
<td>MeOH</td>
<td>86:14</td>
<td></td>
</tr>
</tbody>
</table>

* Reactions conducted at room temperature and 1 atm, using a concentration of 0.8% Raney Ni as a 50% w/w slurry in H2O (pH = 10). b 1% w/w of catalyst. c Determined by NMR. d Concentration = 10%.

of the cyclization process is not affected by the temperature or the nature of the halogen (for 8a: 70% yield after 15 min at 40 °C and 72% yield at 0 °C; for 8b: 69% yield after 15 min at 0 °C). This fact can be expected taking into account the fast reaction rate. The reaction yield decreases as a result of the competing basic hydrolysis (aminolysis) of the Boc and benzoyl groups promoted by the base (KHMDS). The resulting amino alcohol is very soluble in water and is lost during work up. This problem is overcome when protected prolinol 9 is not isolated (vide infra).

Various attempts to deprotect the benzoyl group of 9 under basic conditions (NaOH) led to mixtures of compounds in which cleavage of the Boc group was observed. The problem was solved by hydrolyzing both the Boc and the benzoyl groups under acidic conditions (1 N HCl, 100 °C). The crude amino alcohol hydrochloride obtained was converted quantitatively into Fmoc-protected amino alcohol 10 under Schotten–Baumann conditions. Compound 10 can be obtained directly from 8a (without isolation of 9) in 88% yield. Finally, oxidation of the hydroxymethyl moiety of 10 to carboxylic acid with Jones reagent in acetone provided Fmoc-protected trans-4-methylproline (dr = 95:5, ee = 96%).
Visualization was accomplished with UV light, iodine or 1% KMnO₄ in H₂O. Flash chromatography was performed using Merck silica gel 60 (40–63 μm). The detector wavelength, flow rate and solvents were as denoted. The retention times (tᵣ) for the enantiomers are reported.

Solvents were dried using standard procedures.¹⁶ 8-Serine was purchased from Fluka. DiBAL-H (1 M in toluene) was obtained from aluminium metal and KHMDS (0.5 M in toluene) obtained from Fluka. 10% Pt/C, Pt₂O, and Raney Nickel (50% w/w slurry in H₂O, pH = 10) were purchased from Aldrich and KHMDS (0.5 M in toluene) was obtained from Aldrich and KHMDS (0.5 M in toluene). The solvent was washed with H₂O (2 × 50 mL) and the aqueous layer was dried (MgSO₄) and filtered and the solvent removed under reduced pressure.

In a 1 L three-necked round-bottomed flask under argon was placed (c-carboximethylidene)triphenylphosphorane (38.6 g, 110 mmol, 110 mol %) in dry CH₂Cl₂ (500 mL) and stirred at room temperature for 1 h and filtered through a pad of Celite, and the solvent was evaporated under reduced pressure. The residue was taken up in Et₂O (100 mL) and washed with H₂O (2 × 50 mL). The organic phase was dried (MgSO₄) and filtered and the solvent removed under reduced pressure.

Visualization was accomplished with UV light, iodine or 1% KMnO₄ in H₂O. Flash chromatography was performed using Merck silica gel 60 (40–63 μm). The detector wavelength, flow rate and solvents were as denoted. The retention times (tᵣ) for the enantiomers are reported.

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(25R,4R)-2-Amino-N-[[1,1-dimethyl]ethoxycarbonyl]-1-chloro-4-methylpentan-1-ol (7a). In a 250 mL round-bottomed flask was placed [2R,3S]-1-chloro-2-methyl-3-N-[[1,1-dimethyl]ethoxycarbonyl]-2,2-dimethyl-1,3-oxazolidin-4-yl)propane (3.5 g, 11.9 mmol) of dr = 86:14 in AcOH 80% (70 mL). The mixture was stirred at 50 °C during 1.5 h and evaporated to dryness at reduced pressure (without heating). The residue was washed with Et2O (70 mL) and washed with 1% NaHCO3 (4 × 50 mL). The organic layer was dried (MgSO4) and filtered and the solvent removed under reduced pressure. An oil was obtained (2.8 g, 11.1 mmol, 94%), which was used for further purification: Rf = 0.25 (silica, Hex/AcEt 1:1); [α]D190 = −17.5 (c = 0.76, CHCl3) (dr = 86:14); H NMR (400 MHz, CDCl3) δ 4.71, 4.61 (2 bs, 1H), 3.80 – 3.40 (m, 5H), 2.50 – 2.20 (bs, 1H), 1.96 (m, 1H), 1.70 – 1.58 (m, 1H), 1.45 (s, 9H), 1.44 – 1.30 (m, 1H), 1.07 (d, J = 6.8 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 162.5, 79.7, 65.7, 54.5, 50.2, 35.6, 31.8, 28.4, 18.5 (major diastereoisomer). 156.2, 79.7, 65.5, 40.4, 28.9, 22.3, 17.8 (minor diastereoisomer); HRMS calc for M+ (C12H22NO3Cl) 251.1283, found 251.1280.

(25R,4R)-2-Amino-N-[[1,1-dimethyl]ethoxycarbonyl]-1-bromo-4-methylpentan-1-ol (7b). Following the same procedure described for 7a, with [2R,3S]-1-bromo-2-methyl-3-N-[[1,1-dimethyl]ethoxycarbonyl]-2,2-dimethyl-1,3-oxazolidin-4-yl)propane (280 mg, 0.83 mmol) of dr = 80:20 in AcOH 80% (10 mL), an oil was obtained (225 mg, 0.76 mmol, 91%), which was used for further purification: Rf = 0.35 (silica, Hex/AcEt 1:1); [α]D190 = −14.1 (c = 1.01, CHCl3) (dr = 80:20); H NMR (400 MHz, CDCl3) δ 4.70, 4.63 (2 bs, 1H), 3.80 – 3.30 (m, 5H), 2.25 – 2.15 (bs, 1H), 1.92 (m, 1H), 1.80 – 1.55 (m, 1H), 1.55 – 1.32 (m, 10H), 1.08, 1.07 (2d, J = 6.4 Hz, 3H); 13C NMR (100 MHz, CDCl3) δ 162.5, 79.7, 65.5, 54.5, 50.2, 35.6, 31.8, 28.4, 18.5 (major diastereoisomer). 156.2, 80.2, 66.6, 54.5, 50.6, 36.9, 32.3, 28.4, 17.8 (minor diastereoisomer); HRMS calc for M+ (C12H22NO3Br) 295.0778, found 295.0742.
(25,4R)-trans-N-(9-Fluorenylmethoxycarbonyl)-4-methylproline (11). In a 25 mL two-necked round-bottomed flask was placed (2S,4R)-N-(9-fluorenylmethoxycarbonyl)-2-hydroxymethyl-4-methylpyrrolidine (120 mg, 0.36 mmol) in 10 mL of dry acetone. Jones reagent (0.5 mL) was added dropwise at room temperature, and the mixture was stirred vigorously for 1 h. Acetone was removed under reduced pressure, and the residue was dissolved in AcOEt (15 mL) and washed with H2O (3 x 10 mL). The organic layer was extracted with 5% NaHCO3 (4 x 10 mL) cooled to 0 °C, which was washed with AcOEt (10 mL) and acidified to pH = 2 with 1 N HCl (approx. 20 mL). The product was extracted with CHCl3 (4 x 10 mL), which was washed with brine (2 x 10 mL), dried (MgSO4), and filtered, and the solvent was removed under reduced pressure. An amorphous solid was obtained (75 mg, 0.21 mmol, 59%) (two rotamers): Rf = 0.29 (silica, Hex/AcOEt 1:2); mp = 65–67 °C; [α]D20 = −49.3 (c = 0.3, CHCl3); 1H NMR (400 MHz, CDCl3) δ 9.8–8.8 (bs, 1H), 7.77–7.66 (m, 2H), 7.62–7.51 (m, 2H), 7.45–7.22 (m, 4H), 4.50–4.28 (m, 3H), 4.24, 4.12 (2t, J = 7.2 Hz, 1H rotamers), 3.74 (dd, J = 9.6, 8.0 Hz, 1H), 3.05 (dd, J = 9.6, 9.2 Hz, 1H), 2.50–2.30 (m, 1H), 2.30–2.24 and 2.20–2.10 (2m, 1H rotamers), 1.93–1.76 (m, 1H), 1.07, 1.03 (2d, J = 6.4 Hz, 3H rotamers); 13C NMR (100 MHz, CDCl3) δ 178.0, 176.2, 155.8, 154.4, 144.0, 143.8, 143.7, 143.7, 141.4, 141.3, 141.2, 140.1, 128.3, 127.7, 127.6, 127.4, 127.0, 127.0, 125.7, 125.1, 125.0, 124.9, 124.8, 120.1, 120.0, 119.9, 67.9, 67.4, 59.5, 58.8, 53.6, 53.4, 47.2, 38.5, 36.9, 32.2, 31.0, 17.1; HRMS calcd for M+ (C21H21NO4) 351.1465, found 351.1395.

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Supporting Information Available: Copies of 1H and 13C NMR spectra of compounds 3–11. This material is available free of charge via the Internet at http://pubs.acs.org.