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Practical syntheses of 4-fluoroprolines

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ABSTRACT

4-Fluoroprolines are among the most useful nonnatural amino acids in chemical biology. Here, practical routes are reported for the synthesis of the 2*S*,4*R*, 2*S*,4*S*, and 2*R*,4*S* diastereomers of 4-fluoroproline. Each route starts with (2*S*,4*R*)-4-hydroxyproline, which is a prevalent component of collagen and hence readily available, and uses a fluoride salt to install the fluoro group. Hence, the routes provide process-scale access to these useful nonnatural amino acids.

1. Introduction

The ability to incorporate nonnatural amino acids into proteins is altering the landscape in chemical biology and related fields [1]. Perhaps no other nonnatural amino acid has been as useful in enhancing the conformational stability of proteins as have 4-fluoroprolines. In the proper context, the 4*R* and 4*S* diastereomers of 4-fluoroproline have been shown to increase dramatically the conformational stability of collagen [2], which is the most abundant protein in animals. In addition, 4-fluoroproline residues can endow conformational stability upon other proteins [3] as well as peptides [4]. This enhanced stability is the result of the increased tendency of 4-fluoroproline residues to adopt a C^{γ}-*exo* or C^{γ}-*endo* pyrrolidine ring pucker (2*S*,4*R* or 2*S*,4*S* diastereomer, respectively), or form a *trans* or *cis* peptide bond (2*S*,4*R* or 2*S*,4*S* diastereomer, respectively). These conformational preferences arise from two stereoelectronic effects [5]: a *gauche* effect that fixes the pyrrolidine ring pucker [6,7], and an $n \rightarrow \pi^*$ interaction that stabilizes the *trans* peptide bond [6,8]. Because of these desirable attributes, there is a growing need to access 4-fluoroprolines.

The chemical synthesis of a 4-fluoroproline often begins with a 4-hydroxyproline [9]. (2*S*,4*R*)-4-Hydroxyproline (HypOH), which is a prevalent component of collagen, is an especially advantageous starting material for the synthesis of 4-fluoroprolines. The prevalence of this amino acid is extraordinary: the abundance of Hyp within animal proteins is ~4%, a value calculated from the abundance of collagen amongst animal proteins (1/3) and the prevalence of Hyp within collagen (~38% × 1/3) [10]. Thus, the abundance of Hyp in animals exceeds that of seven "common" amino acids: Cys, Gln, His, Met, Phe, Trp, and Tyr [11].

Typically, the key reaction in synthetic routes to a 4-fluoroproline involves the S_N2 displacement of an activated hydroxyl group in a 4-hydroxyproline by a fluoride ion, resulting in the formation of a C–F bond with the inversion of configuration at C-4. (Diethylamino)-Sulfur trifluoride (DAST [12]) or a congener, such as 4-morpholinosulfur trifluoride (morph-DAST), is typically employed in this strategy [9,13], as such reagents can both activate the hydroxyl group and supply the fluoride ion. DAST and its congeners are, however, both expensive and explosive [14]. Moreover, the analogous precursor of (2*S*,4*R*)-4-fluoroproline – (2*S*,4*S*)-4-hydroxyproline – is itself a nonnatural amino acid that is expensive.

Here, we report on practical synthetic routes to three 4-flouroproline diasteromers: 2*S*,4*R* (**1**), 2*S*,4*S* (**2**), and 2*R*,4*S* (**3**). The routes all start with HypOH, and none use a DAST-like reagent. All are amenable to a process scale.

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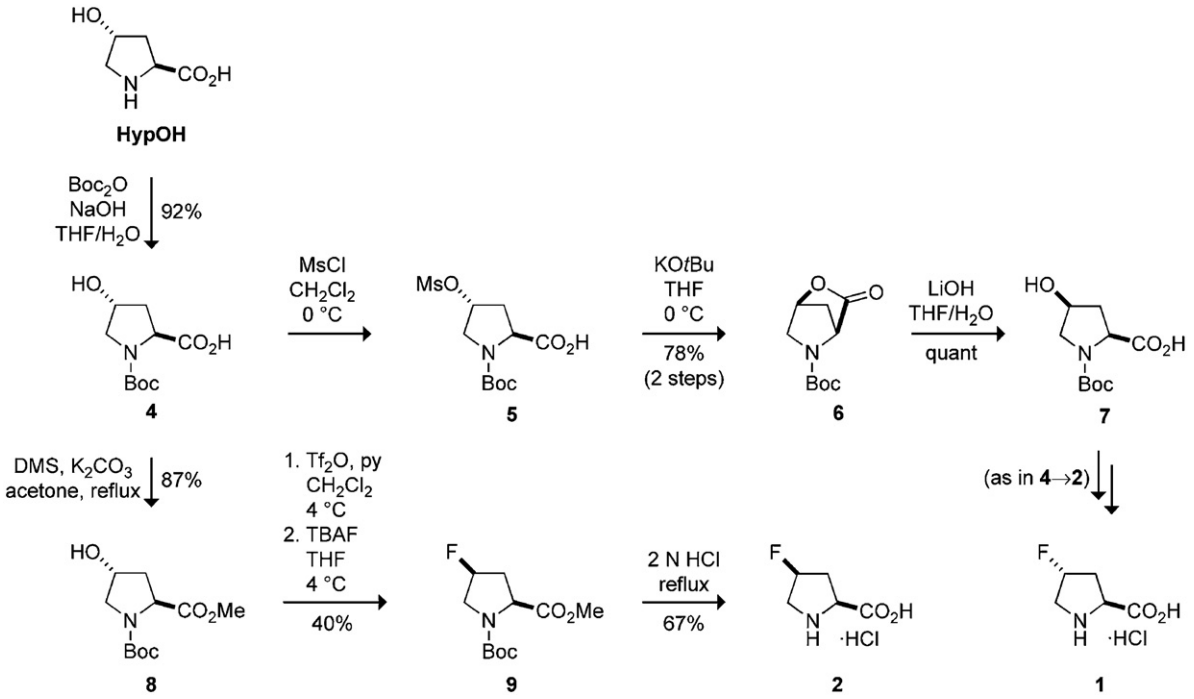


Scheme 1.

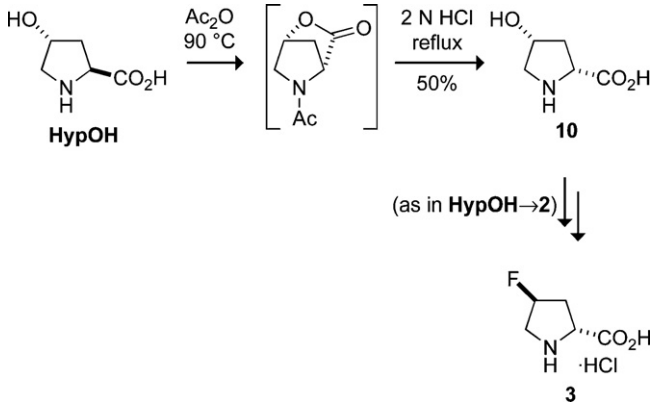
2. Results and discussion

The 2*S*,4*S* diastereomer of 4-fluoroproline (**2**) was synthesized from HypOH by using the S_N2 reaction of a fluoride ion to invert the configuration at C-4. Specifically, (2*S*,4*S*)-4-fluoroproline (**2**) was synthesized in four steps from HypOH with an overall yield of 16%. As shown in Scheme 1, the hydroxyl group of compound **4** was activated with trifluoromethane sulfonic anhydride, and the ensuing triflate was displaced with tetra-*n*-butyl ammonium fluoride (TBAF [15]) to yield protected (2*S*,4*S*)-4-fluoroproline; deprotection with acid gave the 2*S*,4*S* diastereomer of 4-fluoroproline (**2**).

Likewise, key precursors to the 2*S*,4*R* and 2*R*,4*S* diastereomers of 4-fluoroproline (**1** and **3**) were synthesized from HypOH. The routes to these precursors have a notable feature. The route to the 2*S*,4*R* diastereomer epimerizes C-4; the route to the 2*R*,4*S* diastereomer epimerizes C-2. Each of these epimerizations was accomplished with a distinct lactonization reaction.

N-Boc-(2*S*,4*S*)-4-hydroxyproline (**7**) was synthesized in four steps from HypOH with an overall yield of 72%. As shown in Scheme 1, the amino group of HypOH was protected as a Boc carbamate. Mesylation, followed by the formation of lactone and its hydrolysis, inverted the stereochemistry at C-4. Compound **7** can be taken on to (2*S*,4*R*)-4-fluoroproline (**1**) by the same three steps used to convert compound **4** to (2*S*,4*S*)-4-fluoroproline (**2**) with similar yields (data not shown).

(2*R*,4*R*)-4-Hydroxyproline (**10**) was synthesized from HypOH in one step with a yield of 50%. As shown in Scheme 2, HypOH was treated with acetic anhydride to produce (2*R*,4*R*)-4-hydroxyproline lactone. This remarkable transformation has been proposed to proceed via a bicyclic mesoionic intermediate [16], and was used recently in the synthesis of alkaloids from HypOH [17]. The reaction conditions racemize C-2 of other amino acids, but lead to a single enantiomer here because lactonization traps the 2*R* epimer. The lactone was hydrolyzed in acid to yield (2*R*,4*R*)-4-hydroxyproline. Compound **10** can be taken on to (2*R*,4*S*)-4-fluoroproline (**3**) by the same four steps used to convert HypOH to (2*S*,4*S*)-4-fluoroproline (**2**) with similar yields (data not shown).



Scheme 2.

3. Conclusion

We have described practical routes to the 2*S*,4*R*, 2*S*,4*S*, and 2*R*,4*R* diastereomers of 4-fluoroproline. These routes, which we have used to synthesize 4-fluoroprolines on a kilogram scale, provide ready access to a nonnatural amino acid of growing importance to chemical biology. Finally, we note that these routes accommodate the large-scale synthesis of [F-18]4-fluoroprolines. These probes, along with positron emission tomography, have proven to be useful for monitoring the healing of wounds, permeability of the blood–brain barrier, and other physiological processes [18].

4. Experimental

4.1. Synthesis of N-Boc-(2S,4S)-4-hydroxyproline (7)

4.1.1. N-Boc-(2S,4R)-4-hydroxyproline (4)

HypOH (15.0 g, 0.114 mol) was dissolved in 150 mL of 2:1 THF/H_2O. To this solution were added 50 mL of 10% (w/v) NaOH(aq) and

Boc anhydride (30.0 g, 0.137 mol), and the resulting solution was stirred at room temperature overnight. The THF was removed by rotary evaporation under reduced pressure. The residue was dissolved in ethyl acetate, and the pH was adjusted to 2 by the addition of 10% (w/v) $KHSO_4$(aq). The acidic solution was extracted several times with ethyl acetate. The combined organic extracts were washed with H_2O and brine, and then dried over anhydrous Na_2SO_4(s). The desiccant was removed by filtration, and the solvent was removed by rotary evaporation under reduced pressure to give **4** (24.7 g, 92%) as a syrup.

4.1.2. N-Boc-(2S,4R)-4-mesylproline (5)

Compound **4** (2.00 g, 8.65 mmol) was dissolved in CH_2Cl_2 at 0 °C. Pyridine (1.40 mL, 17.3 mmol) and mesyl chloride (1.33 mL, 17.3 mmol) were added, and the resulting solution was stirred overnight. The reaction was quenched with water, and the aqueous layer was extracted with CH_2Cl_2 (3×50 mL). The combined extracts were washed with water and brine, dried over anhydrous Na_2SO_4(s), and evaporated to dryness. The crude product **5** was taken on without purification. (The characterization of compound **5** was as described by Schäfer and coworkers [19].)

4.1.3. N-Boc-(2S,4S)-4-hydroxyproline lactone (6)

Compound **5** (2.80 g, 9.09 mmol) was dissolved in THF containing KO*t*Bu (1.22 g, 10.9 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 12 h. After the reaction was complete (as monitored by TLC), the reaction mixture was quenched with water and extracted with ethyl acetate (2×50 mL) to furnish **6** (1.46 g, 78% over two steps) as a syrup. (The characterization of compound **6** was as described by Silverman and coworkers [20].)

4.1.4. N-Boc-(2S,4S)-4-hydroxyproline (7)

Compound **6** (1.00 g, 4.69 mmol) was dissolved in 35 mL of 2:2:3 THF/MeOH/H_2O. LiOH (0.33 g, 14.1 mmol) was added, and the resulting solution was stirred overnight. The organic solvent was removed by rotary evaporation under reduced pressure. The residue thus obtained was dissolved in 25 mL of ethyl acetate, and the resulting solution was acidified with a saturated aqueous solution of $KHSO_4$. The aqueous layer was extracted with ethyl acetate (2×50 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4(s) and concentrated under reduced pressure to yield **7** (1.09 g, quant) as a syrup.

4.2. Synthesis of (2S,4S)-4-fluoroproline (2)

4.2.1. N-Boc-(2S,4R)-4-hydroxyproline methylester (8)

Compound **4** (10.0 g, 46.3 mmol) was dissolved in 60 mL of anhydrous acetone. Dimethylsulfate (7.60 mL, 78.7 mmol) and K_2CO_3 (19.0 g, 138.9 mmol) were added, and the resulting solution was heated at reflux for 6 h. The solution was then cooled to room temperature, filtered through a pad of Celite®, and concentrated under reduced pressure. Chromatography (silica gel, 1:1 ethyl acetate/petroleum ether) afforded **8** (9.88 g, 87%) as a solid. (The characterization of compound **8** was as described by Fang and coworkers [21]).

4.2.2. N-Boc-(2S,4S)-4-fluoroproline methylester (9)

Compound **8** (2.98 g, 12.2 mmol) was dissolved in 100 mL of dry methylene chloride. This solution was cooled to 4 °C, and 30 mL of dry pyridine was added, followed by 3.1 mL of trifluoromethane sulfonic anhydride. The solution was allowed to stir for 30 min and then washed with 1N HCl. The aqueous phase was extracted with methylene chloride (2×50 mL), and the combined organic extracts were dried over $MgSO_4$(s) and concentrated under reduced pressure. The reddish syrup thus obtained was dissolved in 100 mL of THF and cooled to 4 °C. To this solution was added a 1.0 M solution of TBAF in THF (1.15 g, 2.5 eq). The resulting mixture was stirred at 4 °C overnight, and then concentrated under reduced pressure. Chromatography (silica gel, 1:1 ethyl acetate/petroleum ether) furnished **9** (1.21 g, 40%) as a solid. (The characterization of compound **9** was as described by Chiba et al. [22]).

4.2.3. (2S,4S)-4-Fluoroproline (2)

Compound **9** (2.00 g, 8.09 mmol) was dissolved in 10 mL of 2N HCl. The resulting solution was heated under reflux for 2–4 h. After the reaction was complete (as monitored by TLC), the solution was decolorized with charcoal while still hot, and filtered through a pad of Celite®. Water was removed by rotary evaporation under reduced pressure and as an azeotrope with dry toluene. Crystallization (1:1 ethyl acetate/petroleum ether) afforded the hydrochloride salt of **2** (0.67 g, 49%) as a white solid.

4.3. Synthesis of (2S,4S)-4-hydroxyproline (10)

A stirred mixture of HypOH (6.55 g, 50.0 mmol) in 40 mL of acetic anhydride was heated at 90 °C for 7 h under N_2(g). Solvent was removed under reduced pressure. The thick oil thus obtained was dissolved in 25 mL of 2N HCl, and the resulting solution was heated at reflux for 3 h. The pH of the solution was then adjusted to 6 by the addition of NaOH(aq), water was removed under reduced pressure, and the product was isolated and purified by crystallization (1:1 ethyl acetate/petroleum ether) to yield **10** (3.27 g, 50%) as a solid. (The characterization of compound **10** was as described by Dalla Croce and La Rosa [16].)

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