Stereocontrolled Synthesis of (*S*)-γ-Fluoroleucine

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Abstract: Starting with (*S*)-leucine, the corresponding γ-fluoride **8** has been prepared in a stereocontrolled fashion, by exploiting methods for the direct side-chain bromination of amino acid derivatives and silver(I) fluoride as the fluorinating reagent.

Key words: (*S*)-γ-fluoroleucine, stereoselective synthesis, silver fluoride, amino acids, halogenation

Fluorinated amino acids and their derivatives are of interest as mechanistic[1] and spectroscopic probes,[2] and as enzyme inhibitors[3] and in the development of pharmaceuticals.[4] Consequently they have attracted considerable attention as synthetic targets, with particular emphasis on asymmetric syntheses and methods to introduce the fluorine under straightforward conditions.[4,5] We are exploring the incorporation of aliphatic fluorinated amino acids into proteins by cell-free protein synthesis.[6] In this context, (*S*)-γ-fluoroleucine (**8**) presents a particularly attractive probe because of the simplicity of its spin system and because leucine **1** is an abundant amino acid in proteins, both at positions inside the protein structure and on the protein surface. (2*S*,4*S*)-5-Fluoroleucine has already been shown to be incorporated into proteins made by *E. coli*.[7] In 1994, Papageorgiou et al.,[8] reported the synthesis of the ethyl ester of (*S*)-γ-fluoroleucine (**8**), using Schöllkopf's bislactim ether methodology and starting by treating isobutylene oxide with HF·pyridine. They noted briefly that saponification of the ester with $Ba(OH)_2$ afforded the free amino acid **8**. Another synthesis of the ester, also starting with isobutylene and HF·pyridine, and with lipase-catalyzed resolution of a racemic intermediate, was reported in 2005.[9] A complementary approach is to begin with a chiral amino acid, where the stereochemical center of the starting material is retained in the product. In that context, Truong et al.[10] recently reported the synthesis of the trifluoroacetate salt of the amino acid **8** in eight steps from *N*-(*tert*-butoxycarbonyl)-(*S*)-aspartic acid 4-benzyl ester, using DAST in a key step to introduce the fluorine. This publication prompts us to report our stereocontrolled synthesis of the amino acid **8** from (*S*)-leucine (**1**), by exploiting methods for the direct side-chain bromination of amino acid derivatives[11] and AgF as the fluorinating reagent.

Treatment of (*S*)-leucine (**1**) with phthalic anhydride and Et_3N in refluxing toluene, and then reaction of the corresponding phthalimide with $SOCl_2$-pretreated methanol, afforded the amino acid derivative **2**, which reacted with NBS to give the bromide **3**.[11] The amino acid protecting groups and the brominating reagent were carefully selected to control the regioselectivity of the halogenation. The solvent reported for the latter reaction is CCl_4 but trifluoromethylbenzene is generally more acceptable[12] and proved to be a suitable alternative for this purpose. Reaction of the bromide **3** with AgF gave the fluoride **6**, which had to be separated from the byproducts, the lactone **4** and the alkene **5**, through chromatography. Despite a comprehensive investigation of all the reaction variables, including temperature and time, and relative and absolute concentrations of the reagent and the substrate **3**, formation of the byproducts was unavoidable. All attempts to remove both of the protecting groups from the fluoride **6** in a single procedure failed to afford the free analogue **8**, with most protocols instead producing the lactones **4** and **9**. However, treatment of the fluoride **6** with hydrazine gave the hydrazide **7**, from which the hydrobromide salt of (*S*)-γ-fluoroleucine (**8**) was obtained, in >95% ee, by treatment with NBS.[13] The yield of the crude product was virtually quantitative, but isolation and purification of the free amino acid **8** by treatment with propylene oxide in ethanol was accompanied by decomposition to the lactone **9**, such that the pure zwitterion **8** was obtained in only 45% yield. We have included full details of our experimental protocols and spectral data for the synthesis and characterization of the fluoride **8**,[14] since neither of the previous reports[8,10] provided that information.

In summary, the synthesis outlined in Scheme 1 provides ready access to gram quantities of (*S*)-γ-fluoroleucine (**8**), in only five steps, using convenient protocols and the cheap and readily available starting material **1**, while avoiding the use of toxic and corrosive reagents such as HF·pyridine and DAST, and the need to resolve stereoisomers. It further illustrates the utility of side-chain functionalization and manipulation of amino acid derivatives in stereocontrolled synthesis. Our preliminary experiments have shown that both the hydrazide **7** and the free amino acid **8** are incorporated in place of leucine **1** during cell-free protein synthesis, an intriguing result that is the subject of on-going investigations.

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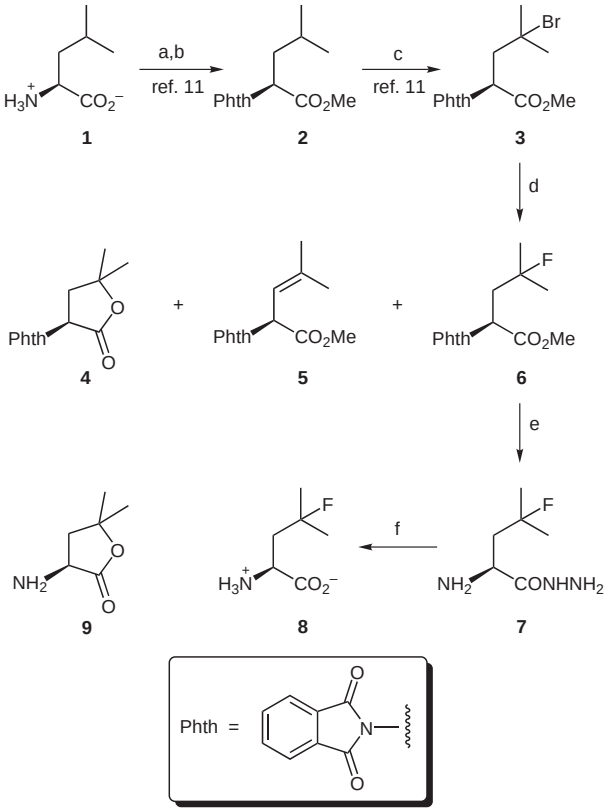

Scheme 1 *Reagents and conditions:* a) phthalic anhydride, toluene at reflux, 25 min; b) $SOCl_2$–MeOH, 25 °C, 12 h; c) NBS, CCl_4 or CF_3Ph, *hν*, reflux, 5 h; d) AgF, MeCN, 25 °C, 22 h; e) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 1 h; f) NBS, H_2O, 25 °C, 0.5 h.

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(14) **Preparation of the Fluoride 6**
A mixture of the bromide **3**[11] (15 g, 42 mmol) and AgF (53 g, 0.42 mol) in dry MeCN (0.25 L) was stirred at 25 °C for 22 h, before it was filtered through silica. The filtrate was concentrated under reduced pressure and the residue was chromatographed on silica, eluting with Et_2O–hexane (1:1, v/v), to give the fluoride **6** (3.7 g, 30%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ = 1.36 (3 H, d, *J* = 25 Hz), 1.43 (3 H, d, *J* = 25 Hz), 2.62 (2 H, m), 3.73 (3 H, s), 5.15 (1 H, m), 7.72–7.79 (4 H, m). ^{13}C NMR ($CDCl_3$): δ = 170.1 (s), 167.9 (s), 134.5 (s), 132.2 (s), 123.9 (s), 94.8 (d, *J* = 167 Hz), 53.4 (s), 48.6 (s), 38.9 (d, *J* = 21 Hz), 28.3 (d, *J* = 24 Hz), 25.8 (d, *J* = 25 Hz).
Preparation of the Hydrazide 7
A mixture of the fluoride **6** (2 g, 6.8 mmol)and $N_2H_4{\cdot}H_2O$ (2.9 mL, 59 mmol) in EtOH (30 mL) was heated at reflux for 1 h, before it was cooled and filtered. The filtrate was concentrated under reduced pressure to give the hydrazide **7** (0.96 g, 86%) as a colorless solid, mp 78–80 °C. ^{1}H NMR (TFA–D_2O): δ = 1.46 (3H, d, *J* = 23 Hz), 1.48 (3 H, d, *J* = 22 Hz), 2.29 (2 H, m), 4.40 (1 H, m). ^{13}C NMR (TFA–D_2O): δ = 171.0 (s), 99.2 (d, *J* = 162 Hz), 51.7 (s), 43.6 (d, *J* = 21 Hz), 29.5 (d, *J* = 23 Hz), 27.3 (d, *J* = 23 Hz).
Preparation of (*S*)-γ-Fluoroleucine 8
A solution of the hydrazide (0.89 g, 5.5 mmol) in H_2O (3 mL) was added dropwise over 0.5 h to a vigorously stirred solution of NBS (1.9 g, 11 mmol) in H_2O (2 mL) at 25 °C, then the mixture was concentrated under reduced pressure. The residue was dissolved in dry EtOH (14 mL) and propylene oxide (1.8 mL) was added, before the mixture was let stand at 5 °C for 24 h. The resultant precipitate was separated by filtration to give the fluoride **8** (0.37 g, 45%) as colorless granules, in >95% ee [HPLC t_R = 10.9 min, using a Daicel Chemical Industries Ltd. Crownpak® CR(+) column, 150 mm × 4 mm I.D., eluting at 0.2 mL min^{-1} with 10 mM aq $HClO_4$, compared to a racemic sample under which conditions the *R*-enantiomer had t_R = 9.2 min], mp 200–202 °C. $[\alpha]_D$ –19.2° (*c* 0.040, MeOH). ^{1}H NMR (D_2O): δ = 1.30 (3 H, d, *J* = 23 Hz), 1.32 (3 H, d, *J* = 23 Hz), 2.09 (2 H, m), 3.81 (1 H, m). ^{13}C NMR (D_2O): δ 177.3 (s), 100.4 (d, *J* = 160 Hz), 54.6 (s), 43.7 (d, *J* = 21 Hz), 30.2 (d, *J* = 24 Hz), 27.0 (d, *J* = 24 Hz).