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Efficient synthesis of (2*R*,3*S*)-2-amino-3-(benzyloxy)-4,4,4-trifluorobutanoic acid (4,4,4-trifluoro-OBn-D-*allo*threonine)

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ABSTRACT

An efficient synthesis of the non-proteinogenic amino acid (2*R*,3*S*)-4,4,4-trifluoro(OBn)-threonine is described. Starting with commercially available (*S*)-Garner's aldehyde, the desired amino acid was prepared as its hydrochloride salt in five steps and an overall yield of 33% (59% based on recovered starting material). The utility of this unusual amino acid was demonstrated by its elaboration into a potent and selective androgen.

Incorporating fluorine into molecules of pharmacological interest has played a remarkably important role in drug design and discovery.[1] Despite its size similarity to hydrogen, fluorine varies significantly from hydrogen with regard to its electronegativity, hydrophobicity, hydrogen-bond acceptor capability (when bonded to carbon), and resistance to metabolism. Not surprisingly, incorporating fluorine into otherwise natural biomolecules can be particularly interesting. Beyond the utility of such compounds per se, fluorinated biomolecules can also be powerful extensions to the extant chiral pool. Many fluorinated analogs of otherwise proteinogenic amino acids have been prepared and studied in a variety of contexts. In the course of our own research, we needed to access useful quantities of the O-protected, non-proteinogenic amino acid (2*R*,3*S*)-2-amino-3-benzyloxy-4,4,4-trifluorobutanoic acid hydrochloride (Fig. 1).

There have been a few syntheses of (2*R*,3*S*)-2-amino-3-hydroxy-4,4,4-trifluorobutanoic acid or its enantiomer (2*S*,3*R*)-2-amino-3-hydroxy-4,4,4-trifluorobutanoic acid reported in the literature. For example, the racemic and non-diastereoselective aldol-condensation between a glycine–Schiff-base condensate and trifluoroacetaldehyde has been reported. The diastereomers were then separated by column chromatography, the products acetylated, and the enantiomers resolved by a lipase-mediated saponification.[2] Seebach has reported that aldol reaction between a chiral imidazolidinone and trifluoroacetaldehyde proceeds with good enantioselectivity and moderate diastereoselectivity.[3] It has also been shown that the desired amino acid can be prepared by aldol reaction of a glycine, chiral Schiff-base condensate, and trifluoroacetaldehyde.[4] Additionally, a 'from the ground-up' approach comprising a Sharpless asymmetric dihydroxylation to an olefin and significant further elaboration to yield the desired amino acid has been described.[5]

We either perceived or directly experienced challenges in proceeding with the literature routes, especially in view of our desire to obtain the O-protected amino acid in gram quantities. Our alternate route is shown below (Scheme 1). We started with the commercially available (*S*)-Garner's aldehyde **1** and thus were able to efficiently leverage the extant chiral pool. We added the trifluoromethyl anion equivalent generated by $TMSCF_3$ and TBAF to give **2** as a mixture of two diastereomers in an approximate ratio of 9:1.[6] After benzylation and column chromatography, the major and desired stereoisomer **3** was obtained.[7] Treatment of **3** with *p*-TsOH gave the alcohol **4** in 90% yield. Jones oxidation of **4**, followed by removal of the Boc group using saturated AcOEt with hydrogen chloride provided **5** as a white solid in 84% yield over two steps.

Having developed a scheme that could produce this unusual amino acid in synthetically useful quantities, we proceeded to incorporate the molecule into our target structure **7** (Fig. 2). We were curious to see whether the alteration in hydrophobicity and hydrogen acidity compared to the non-fluorinated structure **8** could affect its activity at our biological target, the androgen receptor.[7a]

The synthesis of **7** is shown in Scheme 2. The key step in the scheme is an ipso-fluoro substitution by the amino acid **6**, affording the adduct **10** in modest yield. Intermediate **10** was taken forward by sequentially coupling with the acyl hydrazide **11**, dehydration with TPP/I_2,[8] and deprotection with BBr_3 to yield the desired product **7**. The binding affinity of compound **7** for the androgen receptor was assayed and determined to have an IC_{50} of 290 nM, indicating a significantly lower affinity than that previously obtained for the non-fluorinated analog compound **8**

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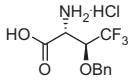
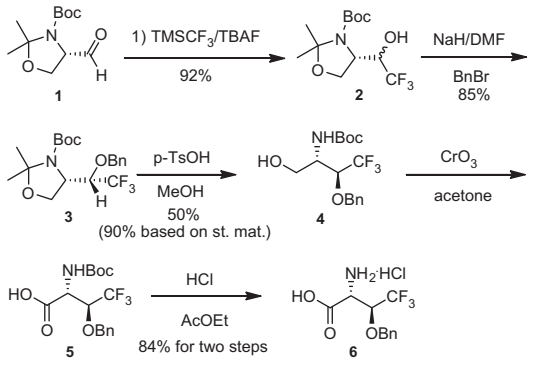


Figure 1. (2*R*,3*S*)-2-Amino-3-benzyloxy-4,4,4-trifluorobutanoic acid hydrochloride.

(0.7 nM).[9] Surprisingly, despite its relatively low binding affinity, compound **7** demonstrated significant in vivo activity in the Rat Herschberger assay.[10] At a dose of just 3 mg/kg (orally), compound **7** increased levator ani muscle ('LABC') in a castrated rat to a level significantly above the non-castrated control ('Sham') and testosterone propionate-treated ('TP') controls (Fig. 3). In contrast, compound **7** increased the weight of the prostate to a significantly lesser degree than that observed for the testosterone-treated or sham control (Fig. 3). Selectivity of anabolic action on muscle over androgenic action on prostate is considered a hallmark of tissue-selective androgens, often referred to as selective androgen receptor modulators (SARMs).

The androgen receptor is a nuclear hormone receptor. While affinity of a ligand for its cognate nuclear receptor is an important first requirement, the ultimate biological activity of the ligand–receptor complex is multi-factorial due to the interaction of the ligand–complex with various co-regulatory proteins that can modulate the transactivation efficacy of the ligand. Further, in vivo results are also dependent on numerous additional variables such as solubility, absorption, and metabolism which might decrease or increase a given molecule's final efficacy and potency. As mentioned previously, because fluorine can affect a number of physical parameters, the ultimate biological results from fluorine substitution are often not predictable a priori. In the instant case, the incorporation of fluorine atoms into a compound with high androgen receptor binding affinity resulted in a compound with low receptor affinity but potent in vivo activity.

In conclusion, we found this chemistry to be a powerful tool for our purposes and thought it worth sharing. The other enantiomer of the described compound could be prepared by starting with (*R*)-Garner's aldehyde. We think it would be interesting to see whether our described method could be used to readily obtain the other diastereomer as well. While a small amount could be available as the minor diastereomer from the extant scheme, we believe that a more productive approach might include a direct inversion at the hydroxyl center, perhaps via a Mitsunobu-type hydroxyl inversion. In this way, the presently described chemistry could be readily expanded to provide all four possible stereiosomers.



Scheme 1. Stereoselective synthesis of (2*R*,3*S*)-4,4,4-trifluoro(OBn)-threonine from (*S*)-Garner's aldehyde in five steps.

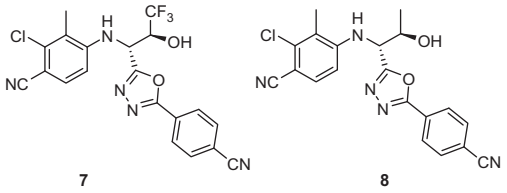
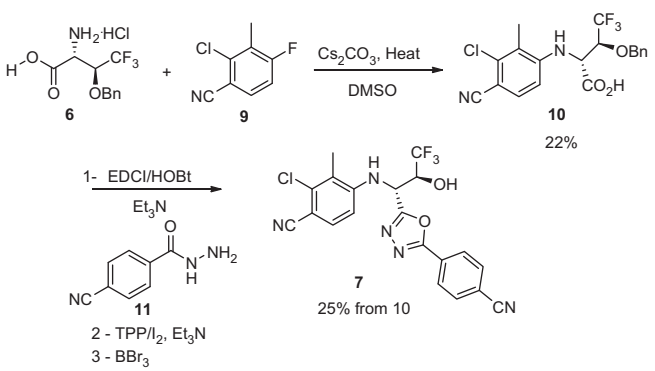


Figure 2. Structures of target compound **7** and non-fluorinated analog **8**.



Scheme 2. Synthesis of non-steroidal androgen **7** from non-proteinogenic amino acid **6**.

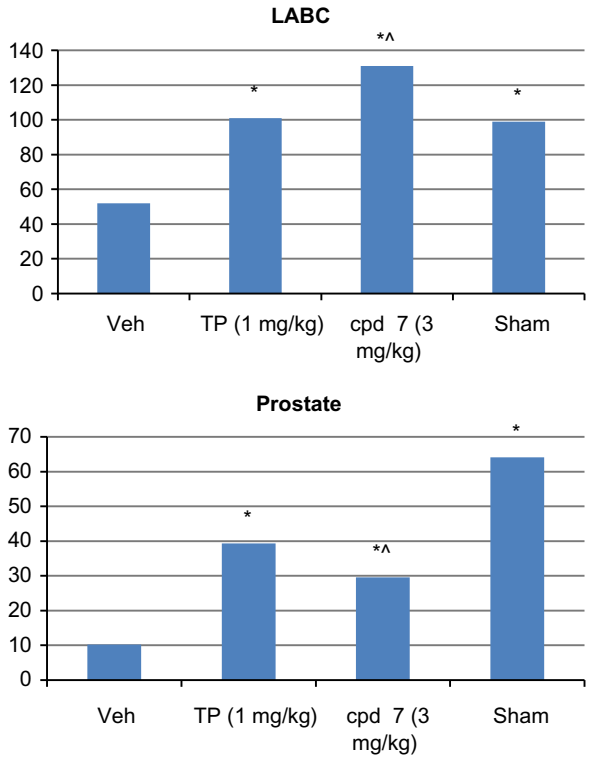


Figure 3. Effect of compd **7** on levator ani muscle (LABC) and prostate weight in treated, castrated rats. Organ and muscle weight is on *Y*-axis and the numbers refer to weight in milligrams. $^{*}\rho$ <0.05 compared to vehicle. ^ρ <0.05 compared to TP and sham.

Acknowledgment

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References and notes

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6. The addition of a trifluoromethyl anion to Garner's aldehyde has been described but the diastereomer ratio was not reported. See Qing, F.-L.; Peng, S.; Hu, C.-M. *J. Fluor. Chem.* **1998**, *88*, 79–81.
7. Experimental procedures and selected analytical data for the preparation of (2*R*,3*S*)-4,4,4-trifluoro(OBn)-threonine.
   Compound **2**: To a mixture of (*S*)-4-formyl-2,2-dimethyl-oxazolidine-3-carboxylic acid *tert*-butyl ester (*S*-Garner's aldehyde) (1.0 g, 4.36 mmol) and (trifluoromethyl)trimethylsilane (2.0 M solution in THF, 2.6 mL, 5.20 mmol) in THF (10 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 0.1 mL, 0.10 mmol) at 0 °C. After addition, the mixture was stirred at room temperature for a weekend (2.5 days). Then, tetrabutylammonium fluoride (1.0 M solution in THF, 9 mL, 9.0 mmol) was added, the mixture was stirred for another 6 h, then quenched by adding saturated aq $NaHCO_3$ solution and extracted with AcOEt. The AcOEt extracts were washed with saturated aq $NaHCO_3$ solution, water, brine and dried over Na_2SO_4. Concentration and purification gave (*S*)-*tert*-butyl 2,2-dimethyl-4-(2,2,2-trifluoro-1-hydroxyethyl)oxazolidine-3-carboxylate (1.21 g, 92%), which showed a complicated NMR spectrum because of the different conformers.
   Compound **3**: To a suspension of NaH (60% in mineral oil, 300.0 mg, 7.50 mmol) in DMF (20 mL) was added (*S*)-*tert*-butyl 2,2-dimethyl-4-(2,2,2-trifluoro-1-hydroxyethyl)oxazolidine-3-carboxylate (1.09 g, 3.64 mmol) in DMF (20 mL), the mixture was stirred at room temperature for 1 h. Then, benzyl bromide (0.86 mL, 7.23 mmol) was added and the mixture was stirred at room temperature overnight, then quenched with ice-water and extracted with AcOEt. The AcOEt extracts were washed with water, brine and dried over Na_2SO_4. Removal of the solvent gave a residue, which was purified by silica gel chromatography to give (*S*)-*tert*-butyl 4-((*R*)-1-(benzyloxy)-2,2,2-trifluoroethyl)-2,2-dimethyloxazolidine-3-carboxylate (1.21 g, 85%), which showed a complicated NMR spectrum because of the different rotamers. ^{1}H NMR (400 MHz, $CDCl_3$) 7.28–7.33 (m, 5H), 4.60–4.85 (m, 3H), 3.93–4.23 (m, 3H), 1.42–1.65 (m, 15H).
   The assignment of the stereochemistry of the major isomer **3** was based on (1) the preferential formation of the anti-adduct from a nonchelation controlled addition of an α-substituted aldehyde and a carbanion;[11] (2) the reported addition of trichloromethyl anion to a very similar aldehyde as Garner's aldehyde gave the anti-adduct as the only diastereomer;[12] and (3) a comparison of ^{1}H NMR spectrum of the amino acid derived from our debenzylated product (the free amino acid) to the data for the same diastereomer as reported in the literature. In particular, the chemical shift of the β-hydrogen (*CH*CF_3) of the anti-isomer as obtained by us at δ 4.24 (DMSO-d_6) closely matches the reported value of δ 4.20 (DMSO-d_6) whereas the chemical shift of the β-hydrogen (*CH*CF_3) of the *syn*-isomer was reported at δ 4.70 (DMSO-d_6).[13]
   Compound **4**: To a solution of (*S*)-*tert*-butyl 4-((*R*)-1-(benzyloxy)-2,2,2-trifluoroethyl)-2,2-dimethyloxazolidine-3-carboxylate (1.21 g, 3.1 mmol) in methanol (100 mL) was added *p*-toluenesulfonic acid monohydrate (90 mg, 0.47 mmol). The mixture was stirred at room temperature for 5 days, then the methanol was removed and the residue was dissolved with AcOEt. The AcOEt solution was washed with saturated aq. Na_2CO_3 solution, water, brine and dried over Na_2SO_4. Removal of the solvent gave a residue, which was purified by flash chromatography to afford the title compound *tert*-butyl (2*S*,3*S*)-3-(benzyloxy)-4,4,4-trifluoro-1-hydroxybutan-2-ylcarbamate (0.54 g, 50%, 90% based on recovered starting material) and recovered starting material (0.54 g), which can be recycled. Mp 90–92 °C; $[\alpha]_D^{25}$ = −66.9 (*c* 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 5.21 (d, *J* = 7.6 Hz, 1H), 4.87 (d, *J* = 11.1 Hz, 1H), 4.58 (d, *J* = 11.1 Hz, 1H), 4.13 (m, 1H), 4.00 (d, *J* = 11.7 Hz, 1H), 3.83 (m, 1H), 3.65 (d, *J* = 11.7 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) 155.7, 136.2, 129.0, 128.9, 128.7, 125.2 (q), 80.4, 78.1 (q), 76.0, 62.1, 50.7, 28.5; HRMS (ES) calcd for $C_{16}H_{22}F_3NO_4Na$ $[M+Na]^+$ 372.1399, found 372.1403.
   Compound **5**: To a solution of *tert*-butyl (2*S*,3*S*)-3-(benzyloxy)-4,4,4-trifluoro-1-hydroxybutan-2-ylcarbamate (1.47 g, 4.2 mmol) in acetone (80 mL) was added Jone's reagent (8.2 mL) at 0 °C. The mixture was stirred at the same temperature for 3 h until the starting material was completely consumed, then the reaction was quenched by adding isopropanol (5 mL). The reaction mixture was extracted with AcOEt. The AcOEt extracts were washed with water, brine and dried over Na_2SO_4. Removal of the solvent gave the crude (2*R*,3*S*)-3-(benzyloxy)-2-(*tert*-butoxycarbonylamino)-4,4,4-trifluorobutanoic acid (1.56 g), which was used directly for the following reaction. Mp 118–120 °C; $[\alpha]_D^{25}$ = +13.1 (*c* 0.79, MeOH); ^{1}H NMR (400 MHz, CD_3OD) 7.25–7.34 (m, 5H), 4.76 (d, *J* = 10.9 Hz, 1H), 4.70 (d, *J* = 10.9 Hz, 1H), 4.53 (d, *J* = 6.4 Hz, 1H), 4.32 (m, 1H), 1.41 (s, 9H); ^{13}C NMR (100 MHz, CD_3OD) 170.7, 156.1, 136.9, 128.2, 128.1, 128.0, 125.0 (q), 79.8, 76.5 (q), 74.8, 53.1, 27.4; HRMS (ES) calcd for $C_{16}H_{21}F_3NO_5$ $[M+H]^+$ 364.1372, found 364.1376.
   Compound **6**: To a solution of (2*R*,3*S*)-3-(benzyloxy)-2-(*tert*-butoxycarbonylamino)-4,4,4-trifluorobutanoic acid (1.56 g, 4.2 mmol) in AcOEt (30 mL) was added AcOEt saturated with hydrogen chloride (30 mL) at 0 °C. Then, the mixture was stirred at room temperature for 1.5 h. After concentration of the reaction mixture to about 15 mL, the white solid was collected by filtration and washed with AcOEt, dried in vacuum to give the title compound (2*R*,3*S*)-2-amino-3-(benzyloxy)-4,4,4-trifluorobutanoic acid hydrochloride salt (1.06 g, 84% for two steps). Mp 194–196 °C; $[\alpha]_D^{25}$ = −13.8 (*c* 1.25, MeOH); ^{1}H NMR (400 MHz, CD_3OD) 7.32–7.44 (m, 5H), 4.86 (d, *J* = 2.0 Hz, 2H), 4.70 (dq, *J* = 3.6, 6.6 Hz, 1H), 4.50 (d, *J* = 3.6 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) 166.0, 136.0, 128.6, 128.5, 128.4, 123.9 (q), 74.5, 74.2 (q), 52.4; HRMS (ES) calcd for $C_{11}H_{13}F_3NO_3$ $[M-Cl]^+$ 264.0848, found 264.0843.
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9. The AR-binding assay was performed as specified by the manufacturer (Invitrogen, Madison, WI). Briefly, 1 μl of 10 mM compound was added to 500 μl of AR screening buffer in a 1.5 ml eppendorf tube to make a 2×10^{-5} M stock. 10-fold serial dilutions of the test compounds were prepared ranging in concentration from 10^{-5} M to 10^{-12} M. Each dilution was added in triplicate to a black 384-microtiter plate. The test compounds are diluted twofold in the final reaction. 2× AR-Fluormone™ complex was prepared with 2 nM Flourmone AL Green™ and 30 nM AR. 25 μl of 2× complex was aliquoted to each reaction well, such that the final reaction volume was 50 μl per well. Plate was sealed with a foil cover and incubated in the dark at room temperature for 4 h. Polarization values for each well were measured. The polarization values were plotted against the concentration of the test compound. The concentration of the test compound that results in half-maximum shift equals the IC_{50} of the test compound. As a control, a competition curve for R1881(methyltrienolone) was performed for each assay. Curve Fitting was performed using GraphPad Prism® software from GraphPad™ Software Inc.
10. The Herschberger assay looks primarily at the ability of androgens to increase muscle size in an immature, castrated rat. In addition, androgenic effects are looked at primarily by weighing the prostate. Selective compounds will show a greater increase in the levator ani relative to the prostate when compared to testosterone treated, castrated animals or to intact animals that have not been treated. Immature Sprague Dawley male rats were obtained Charles River Laboratories (Stoneridge, NY). All animals were maintained in a temperature and humidity controlled room with a 12 h light: 12 h dark cycle, with ad lib access to food (TD 291615, Teklad, Madison, WI) and water. Rats were anesthetized and orchidectomized (GDX) or sham surgery (SHAM) was performed. After a 7-day recovery period, the animals were randomized according to weight and assigned to treatment groups (n = 5), SHAM, OVX + vehicle, OVX + compd treated. Testosterone propionate (TP 1 mg/kg in 5% DMSO/95% corn oil) was administered by once daily subcutaneous injections, while the compound was dosed in vehicle (0.5% carboxymethylcellulose) and administered by once daily oral gavage. The rats were then dosed once daily for 4 days. All animals were euthanized via carbon dioxide inhalation 24 h after the last dose. The prostate and levator ani and bulbocavernosus (LABC) tissues were removed, weighed and recorded.
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