A Simple and Efficient Method for the Resolution of All Four Diastereomers of 4,4,4-Trifluorovaline and 5,5,5-Trifluoroleucine

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Fluorinated amino acids have recently emerged as a valuable set of pharmacologically active agents.[1] Fluorine-containing amino acids have been shown to act as inhibitors of enzymes[2] and function as antitumor and antibacterial agents.[1d,e] There has been a special interest in trifluoromethyl-containing amino acids in peptide and protein design[3,4] because the replacement of methyl with trifluoromethyl groups is accompanied by a substantial increase in hydrophobicity, owing to the low polarizibility of the fluorine atoms.[5,6] It is therefore anticipated that introduction of trifluoromethyl groups into the hydrophobic core of proteins would impose minor structural perturbation but would result in enhanced stability of the folded form in aqueous solution. We have recently reported a peptide system based on the coiled coil region of the transcriptional activator GCN4 with an unnatural hydrophobic core, prepared by substituting four leucine and three valine residues with 5,5,5-trifluoroleucine (TFL) and 4,4,4-tifluorovaline (TFV), respectively. The resulting coiled coil structure was more resistant to thermal- and denaturant-induced unfolding than was its hydrocarbon counterpart.[7] Similar studies from other laboratories have reported the incorporation of CF_3-containing amino acids into peptides by in vivo methods with enhanced thermal and chemical stability.[8] It must be emphasized, however, that in these studies both TFL and TFV were incorporated into peptides as a mixture of two C_α-*S* diastereomers.[7,8] Enantiomerically pure trifluoromethyl-containing amino acids are highly desirable for peptide synthesis to avoid compositional heterogeneity of samples and for exploration of stereoelectronic[9] and packing effects.

There has been no lack of effort in attempts to prepare enantiomerically pure TFL and TFV.[10] However, practical methods for enantioselective synthesis and optical resolution have been limited by low efficiency or compromised enantiomeric purity of the final products. Reported here is an efficient resolution of all four diastereomers of TFL and TFV. The method as outlined in Scheme 1 is simple and practical. Appropriately derivatized TFL and TFV could be separated into two enantiomeric pairs by flash column chromatography. Subsequent enzymatic deacylation of the *N*-acetyl enantiomeric pairs of amino acids with porcine kidney acylase I delivers all four diastereomers in an optically pure form.[11]

In the course of our study on the synthesis of enantiomerically pure TFV, we found that Boc-protected 4,4,4-trifluorovalinol α-*S* diastereomers could be easily separated by column chromatography on silica gel.[12] This finding encouraged us to develop a resolution scheme for racemic TFV and TFL. As shown in Scheme 2, Boc-TFV **1** was first converted to Boc-trifluorovalinol **2** via esterification of **1** with methyl iodide, followed by reduction of the methyl ester with sodium borohydride in methanol in 73% overall yield for the two steps. The racemic mixture of trifluorovalinols was easily separated into the two enantiomeric pairs **2a** [(2*S*,3*R*) + (2*R*,3*S*)] and **b** [(2*S*,3*S*) + (2*R*,3*R*)] by column chromatography on silica gel using *n*-pentane/ethyl ether (1:1) as the eluant. Although the methyl esters of Boc-TFV **1** are also separable, they are not stable toward racemization in the subsequent reduction step. Oxidation of the hydroxyl groups of **2a** and **b** with PDC in DMF and removal of the Boc-protecting group with 30% trifluoroacetic acid in methylene chloride followed by acylation of the free amino group afforded the *N*-acetyl amino acids **3a** and **b**, respectively. Finally, enzymatic deacylation of **3a** and **b** with porcine kidney acylase I afforded the four diastereomers **4a**−**d**. Only those diastereomers that had an *S* configuration at C_α were deacylated by the enzyme. Removal of the acetyl group from the two C_α-*R* diastereomers was realized by refluxing with 3 N HCl.

This strategy was also applied to the resolution of TFL (Scheme 3). Initially, Boc-TFL **5** was also converted to the corresponding alcohols following the procedure used for Boc-TFV **1**, but we found that the trifluoroleucinols were not separable by column chromatography on silica

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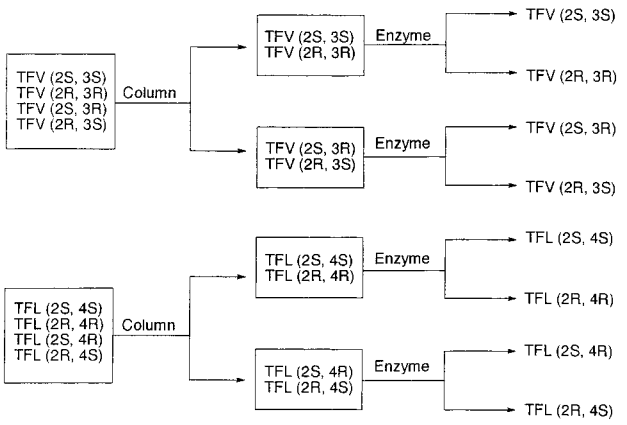
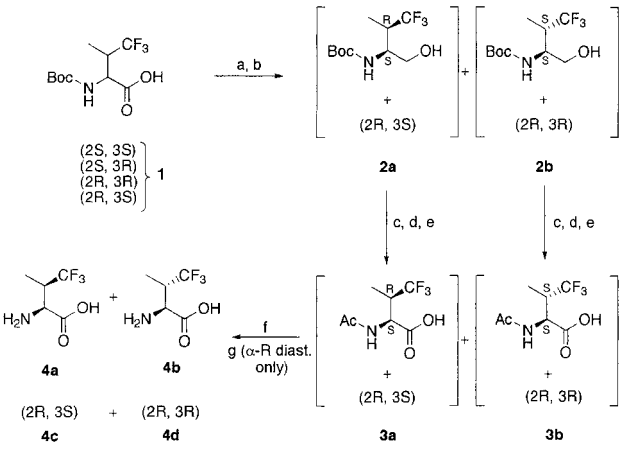
Scheme 1

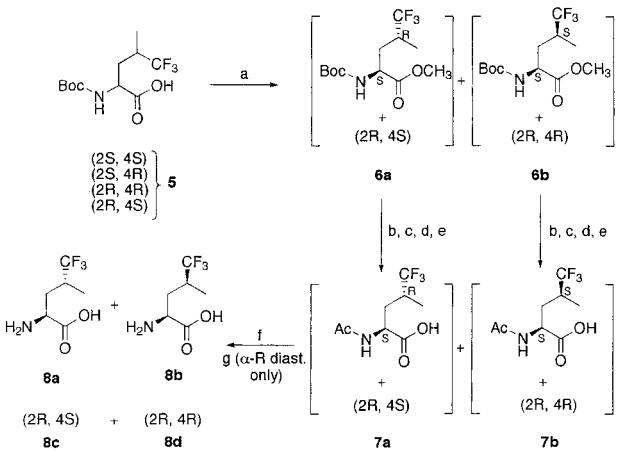


Scheme 2[a]



[a] Reagents and conditions: (a) $NaHCO_3$, CH_3I, DMF, rt, 95%; (b) $NaBH_4$, CH_3OH, 77%; flash column chromatography, *n*-pentane/Et_2O (1:1), silica gel/**2** (300:1); (c) PDC, DMF, rt, 65%; (d) 30% CF_3CO_2H/CH_2Cl_2, 100%; (e) $NaOH/H_2O$, Ac_2O, 0 °C, 95%; (f) porcine kidney acylase I, pH 7.50, 25 °C, 95%; (g) 3 N HCl, 98%.

Scheme 3[a]



[a] Reagents and conditions: (a) $NaHCO_3$, CH_3I, DMF, rt, 95%; flash column chromatography, *n*-pentane/Et_2O (4:1), silica gel/**6** (400:1); (b) $NaBH_4$, CH_3OH, 94%; (c) PDC, DMF, rt, 60%; (d) 30% CF_3CO_2H/CH_2Cl_2, 100%; (e) $NaOH/H_2O$, Ac_2O, 0 °C, 95%; (f) porcine kidney acylase I, pH 7.5, 25 °C, 95%; (g) 3 N HCl, 96%.

gel. Interestingly, the methyl esters of **5** were readily separated into two enantiomeric pairs, **6a** and **b**, on silica gel using *n*-pentane/ethyl ether (3:1) as the eluant and were stable toward racemization in the reduction step. The *N*-acetyl amino acids **7a** and **b** were obtained from **6a** and **b**, respectively, by straightforward functional-group transformations that included reduction of the methyl ester group to hydroxyl, oxidation of the hydroxyl to acid, and replacement of the Boc protecting group with an acetyl group. In the final step, enzymatic deacylation was applied to **7a** and **b** to give diastereomerically pure compounds **8a**−**d**.

The purity of the intermediates and of the final diastereomers was ascertained using ^1H, ^{13}C, and ^{19}F NMR spectroscopy. The ^{19}F NMR technique is particularly useful in this case for purity control because of its high sensitivity and the large chemical-shift dispersion observed for these compounds. The enantiomeric pairs exhibited baseline-separated ^{19}F NMR spectra in each case. Contamination by the other enantiomeric pair or racemization during chemical transformation could be easily detected. The optical purity of the products was also verified by NMR analysis of dipeptides formed by coupling with a side chain-protected methyl ester of L-serine.[3] The ^{19}F NMR spectra clearly showed four peaks for dipeptides derived from the racemic mixture, two peaks for dipeptides derived from enantiomeric pairs, and only one peak for the diastereomerically pure dipeptide.

In summary, we have developed a simple method for efficient resolution of all four diastereomers of TFL and TFV.

Experimental Section

General Procedures. Flash column chromatography was performed on Kieselgel 60 silica gel (230−240 mesh, EM Science) using standard literature procedures.[13] Analytical thin-layer chromatography was performed using E. Merck silica gel Kieselgel 60 F_{254} (0.25 mm) plates. Compounds were made visible by using UV light, by exposure to iodine vapor, or by staining with a ninhydrin solution followed by heating. Reagents and solvents were of reagent grade or better and were obtained from Aldrich Chemical Co., Fluka Chemie AG, Fluorochem USA, Lancaster Synthesis, or Novabiochem Corp. Deuterated solvents were obtained from Cambridge Isotope Laboratories.

Infrared spectra were obtained on a Mattson 1000 FT-IR instrument with a 4 cm^{-1} band-pass. Spectra of solid samples were recorded with either KBr pressed plates or thin layers of organic solutions between NaCl plates. Mass spectra were obtained on a Hewlett-Packard GC−MS (model 5988A) with a dip-probe using conditions as indicated. Nuclear magnetic resonance spectra were recorded on a Bruker AM-300 or a Bruker DPX-300 instrument using standard deuterated solvents. ^{19}F NMR spectra were measured using $CFCl_3$ ($\delta = 0$) for organic solvents and CF_3CO_2H ($\delta = -76.50$) for D_2O as the internal standards. Optical rotations were measured using an AUTOPOL IV digital polarimeter (Rudolph Research Analytical, NJ).

***N*-Boc-4,4,4-trifluorovalinol (2).** To a suspension of Boc-DL-trifluorovaline (1.30 g, 4.79 mmol) and $NaHCO_3$ (1.21 g, 14.37 mmol) in 20 mL of dry DMF was added 0.33 mL of CH_3I (5.27 mmol) at room temperature under argon. The resulting mixture was stirred for 5 h and then partitioned between 75 mL of ethyl acetate and 50 mL of water. The organic layer was washed with water (3 × 50 mL), dried over $MgSO_4$, and concentrated to yield 1.36 g (95%) of the Boc-DL-trifluorovaline methyl ester as a pale-yellow oil.

The Boc-TFV methyl ester (855 mg, 3 mmol) was dissolved in 20 mL of methanol, and $NaBH_4$ (681 mg, 18 mmol) was added in small portions at 0 °C. The reaction mixture was stirred overnight at room temperature and then diluted with 80 mL of ethyl acetate, washed with water (3 × 50 mL), and dried over $MgSO_4$. After removal of the solvent, the crude product (Boc-trifluorovalinol) was chromatographed on a silica gel column (silica gel, 300 g) using *n*-pentane/Et_2O (1:1) as the eluant to

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give 452 mg of **2a** as a pale-yellow solid (58%) and 214 mg of **2b** as a white solid (28%).

(2*S*,3*R*)-(2*R*,3*S*)-*N*-Boc-4,4,4-trifluorovalinol (2a): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.04 (d, 1H, J = 9.3 Hz), 4.02 (m, 1H), 3.62 (m, 3H), 2.61 (m, 1H), 1.44 (s, 9H), 1.15 (d, 3H, J = 7.2 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.20 (C=O), 127.83 (q, CF_3, $^1J_{CF}$ = 279.9 Hz), 80.26 (C), 62.78 (CH_2), 51.09 (CH), 38.47 (q, CH, $^2J_{CF}$ = 25.6 Hz), 28. 40 (3 × CH_3), 8.76 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −70.63 (d, 3F, J = 9.0 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3435s, 3300s, 2990s, 2979m, 2954m, 1691s, 1539s, 1537s, 1265s, 1172s, 1125; GC−MS (CI, CH_4): 258 (14, $[M + 1]^+$), 242 (4), 202 (100), 158 (37), 57 (14); CI-HRMS m/z 258.1322, calcd for $C_{10}H_{19}NO_3F_3$ 258.1317.

(2*S*,3*S*)-(2*R*,3*R*)-*N*-Boc-4,4,4-trifluorovalinol (2b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.11 (d, 1H, J = 8.4 Hz), 3.80 (m, 1H), 3.66 (m, 2H), 3.45 (t, 1H, J = 5.7 Hz), 2.53 (m, 1H), 1.42 (s, 9H), 1.15 (d, 3H, J = 7.2 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.43 (C=O), 127.91 (q, CF_3, $^1J_{CF}$ = 280.2 Hz), 80.30 (C), 62.92 (CH_2), 52.56 (CH), 38.89 (q, CH, $^2J_{CF}$ = 24.8 Hz), 28. 40 (3 × CH_3), 10.59 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −68.76 (d, 3F, J = 8.5 Hz); FT-IR (film, $\tilde{\nu}_{max}$, cm^{-1}) 3436s, 3302s, 3012m, 2990m, 2954m, 1691s, 1532s, 1265s, 1172s, 1127s; GC−MS (CI, CH_4): 258 (14, $[M + 1]^+$), 242 (4), 202 (100), 182 (8), 57 (14); CI-HRMS (NH_3, 170 eV) m/z 258.1314, calcd for $C_{10}H_{19}NO_3F_3$ 258.1317.

(2*S*,3*R*)-(2*R*,3*S*)-*N*-Ac-4,4,4-trifluorovaline (3a).[1f] A solution of alcohol **2a** (257 mg, 1 mmol) in 4 mL of dry DMF was treated with PDC (2.26 g, 6 mmol) at room temperature under argon and stirred overnight. The reaction mixture was then diluted with 20 mL of diethyl ether/30 mL of saturated $NaHCO_3$ solution. The organic layer was washed with 10 mL of saturated $NaHCO_3$. The combined aqueous layers were acidified to pH 2 with 3 N HCl and extracted with diethyl ether (2 × 50 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to yield 176 mg of the corresponding Boc-trifluorovaline[7] (65%).

Boc-TFV (176 mg, 0.65 mmol) was treated with 4 mL of 40% trifluoroacetic acid in CH_2Cl_2 for 10 min. After removal of the solvent, the residue was dissolved in 2 mL of water and treated with NaOH (260 mg, 6.5 mmol) at 0 °C, followed by dropwise addition of acetic anhydride (0.13 mL, 1.3 mmol). The reaction mixture was stirred at 0 °C for 30 min before it was allowed to warm to room temperature. After stirring for another 1.5 h, the mixture was diluted with 10 mL of water, acidified to pH 2 with 1 N HCl, and extracted with ethyl acetate (2 × 60 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to give the desired product **3a** as a white solid (132 mg, 95%). ^{1}H NMR (300 MHz, D_2O) δ 4.96 (d, 1H, J = 3.0 Hz), 3.07 (m, 1H), 2.04 (s, 3H), 1.15 (d, 3H, J = 7.2 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −71.63 (d, 3F, J = 8.8 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3397s (br), 3253s, 3068m, 2981s, 2948m, 1686s, 1552s, 1369s, 1289s, 1174s, 1145s, 1055s; GC−MS (CI, CH_4): 214 (100, $[M + 1]^+$), 196 (9), 172 (33), 82 (33), 57 (6).

(2*S*,3*S*)-(2*R*,3*R*)-*N*-Ac-4,4,4-trifluorovaline (3b):[1f] ^{1}H NMR (300 MHz, D_2O) δ 4.67 (d, 1H, J = 3.3 Hz), 3.07 (m, 1H), 2.04 (s, 3H), 1.17 (d, 3H, J = 7.2 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −69.43 (d, 3F, J = 8.8 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3397s (br), 3253s, 3068m, 2981s, 2948m, 1686s, 1552s, 1369s, 1289s, 1174s, 1145s, 1055s; GC−MS (CI, CH_4): 214 (100, $[M + 1]^+$), 196 (9), 172 (33), 101 (10), 82 (33), 57 (6).

(2*S*,3*R*)-4,4,4-Trifluorovaline (4a).[1f] To a solution of **3a** (107 mg, 0.5 mmol) in 1 mL of pH 7.9 aqueous LiOH/HOAc was added porcine kidney acylase I (10 mg) at 25 °C. The mixture was stirred at 25 °C for 48 h (pH was maintained at 7.5 by periodic addition of 1 N LiOH). The reaction was then diluted with 5 mL of water, acidified to pH 5.0, heated to 60 °C with Norit, and filtered. The filtrate was acidified to pH 1.5 and extracted with ethyl acetate (2 × 10 mL). The aqueous layer was freeze-dried to give 49 mg of **4a** (95%). The combined organic layers were concentrated, and the residue was refluxed in 3 N HCl for 6 h and then freeze-dried to yield 50 mg of **4c** (98%).

The other two diastereomers **4b** and **d** were obtained from **3b** using the same procedure as above.

(2*S*,3*R*)-4,4,4-Trifluorovaline (4a):[1f] ^{1}H NMR (300 MHz, D_2O) δ 4.35 (t, 1H, J = 2.7 Hz), 3.27 (m, 1H), 1.22 (d, 3H, J = 7.5 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −71.69 (d, 3F, J = 9.3 Hz); $[\alpha]^{23.7}_D$ = +7.2° (c 0.75, 1 N HCl).

(2*S*,3*S*)-4,4,4-Trifluorovaline (4b)[1f]**:** ^{1}H NMR (300 MHz, D_2O) δ 4.24 (dd, 1H, J = 2.1, 3.9 Hz), 3.23 (m, 1H), 1.30 (d, 3H, J = 7.2 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −70.04 (d, 3F, J = 9.0 Hz); $[\alpha]^{23.3}_D$ = +12.8° (c 0.5, 1 N HCl).

(2*R*,3*S*)-4,4,4-Trifluorovaline (4c): ^{1}H NMR (300 MHz, D_2O) δ 4.24 (dd, 1H, J = 2.1, 3.9 Hz), 3.23 (m, 1H), 1.30 (d, 3H, J = 7.2 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −70.04 (d, 3F, J = 9.0 Hz).

(2*R*,3*R*)-4,4,4-Trifluorovaline (4d): ^{1}H NMR (300 MHz, D_2O) δ 4.35 (t, 1H, J = 2.7 Hz), 3.27 (m, 1H), 1.22 (d, 3H, J = 7.5 Hz); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −71.69 (d, 3F, J = 9.3 Hz).

***N*-Boc-5,5,5-trifluoroleucine methyl ester (6).** A mixture of Boc-DL-trifluoroleucine (1.25 g, 4.38 mmol), iodomethane (0.3 mL, 4.82 mmol), $NaHCO_3$ (1.1 g, 13.15 mmol), and dry DMF (20 mL) was stirred at room temperature under argon for 6 h and then diluted with 200 mL of ethyl acetate and washed with water (4 × 100 mL). The organic layer was dried over Na_2SO_4 and concentrated to give 1.25 g of the product as a pale-yellow oil (95%). Column chromatography on silica gel (500 g) using Et_2O/*n*-pentane (1:4) as the eluant afforded 420 mg of (2*S*,4*R*)-, (2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucine methyl ester (**6a**) (32%), 347 mg of (2*S*,4*S*)-, (2*R*,4*R*)-*N*-Boc-5,5,5-trifluoroleucine methyl ester (**6b**) (27%), and 337 mg of the mixture of **6a** and **b** (26%).

(2*S*,4*R*)-, (2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucine Methyl Ester (6a): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.29 (d, 1H, J = 6.9 Hz), 4.32 (m, 1H), 3.70 (s, 3H), 2.31 (m, 1H), 2.12 (m, 1H), 1.58 (m, 1H), 1.37 (s, 9H), 1.11 (d, 3H, J = 6.9 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.72 (C=O), 155.29 (C=O), 128.09 (q, CF_3, $^1J_{CF}$ = 278.9 Hz), 80.27 (C), 52.54 (CH_3), 51.70 (CH), 35.13 (q, CH, $^2J_{CF}$ = 26.4 Hz), 32.98 (CH_2), 28.30 (3 × CH_3), 13.17 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −74.15 (d, 3F, J = 8.2 Hz); FT-IR (film, $\tilde{\nu}_{max}$, cm^{-1}) 3360m, 2984m, 2938m, 1747s, 1716s, 1520s, 1368s, 1269s, 1168s, 1133m; GC−MS (CI, CH_4): 300 (2, $[M + 1]^+$), 284 (7), 244 (100), 200 (66), 82 (21), 57 (24); CI-HRMS m/z 300.1402, calcd for $C_{12}H_{21}NO_4F_3$ 300.1423.

(2*S*,4*S*)-, (2*R*,4*R*)-*N*-Boc-5,5,5-trifluoroleucine Methyl Ester (6b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.02 (d, 1H, J = 8.7 Hz), 4.38 (m, 1H), 3.76 (s, 3H), 2.32 (m, 1H), 1.91−1.74 (br m, 2H), 1.44 (s, 9H), 1.20 (d, 3H, J = 6.9 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.03 (C=O), 155.86 (C=O), 128.24 (q, CF_3, $^1J_{CF}$ = 278.9 Hz), 80.57 (C), 52.80 (CH_3), 50.83 (CH), 35.02 (q, CH, $^2J_{CF}$ = 26.9 Hz), 33.00 (CH_2), 28.42 (3 × CH_3), 12.28 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −74.03 (d, 3F, J = 8.7 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3368s, 3014m, 2983s, 2961m, 1763s, 1686s, 1527s, 1265s, 1214s, 1170s, 1053s, 1028s; GC−MS (CI, CH_4): 300 (2, $[M + 1]^+$), 284 (7), 244 (100), 224 (30), 200 (66), 57 (24); CI-HRMS m/z 300.1410, calcd for $C_{12}H_{21}NO_4F_3$ 300.1423.

(2*S*,4*R*)-, (2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucinol. To a solution of **6a** (420 mg, 1.4 mmol) in methanol (10 mL) was added $NaBH_4$ (531 mg, 14.0 mmol) in small portions. The reaction mixture was stirred at room temperature for 1 h before removal of the solvent. The residue was partitioned between 100 mL of ethyl acetate and 50 mL of water. The aqueous layer was extracted with 100 mL of ethyl acetate. The combined organic layers were dried over Na_2SO_4 and concentrated to yield 357 mg of the desired product as a white solid (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.74 (m, 1H), 3.71 (m, 2H), 3.58 (m, 1H), 2.31 (m, 1H), 2.14 (m, 1H), 1.92 (m, 1H), 1.45 (s, 9H), 1.17 (d, 3H, J = 7.0 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.26 (C=O), 128.41 (q, CF_3, $^1J_{CF}$ = 279.4 Hz), 80.14 (C), 64.78 (CH_2), 50.73 (CH), 35.59 (q, CH, $^2J_{CF}$ = 29.6 Hz), 31.74 (CH_2), 28.52 (3 × CH_3), 13.71 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −73.84 (br s, 3F); GC−MS (CI, CH_4): 272 (100, $[M + 1]^+$), 216 (68), 172 (26), 57 (11); CI−HRMS m/z 272.1474, calcd for $C_{11}H_{21}NO_3F_3$ 272.1473.

(2*S*,4*S*)-, (2*R*,4*R*)-*N*-Boc-5,5,5-trifluoroleucinol: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.58 (m, 1H), 3.79 (m, 1H), 3.68 (m, 1H), 3.58 (m, 1H), 2.27 (m, 1H), 2.05 (m, 1H), 1.80 (m, 1H), 1.45 (s, 9H), 1.18 (d, 3H, J = 6.6 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.47 (C=O), 128.56 (q, CF_3, $^1J_{CF}$ = 278.7 Hz), 80.20 (C), 66.31 (CH_2), 49.49 (CH), 35.15 (q, CH, $^2J_{CF}$ = 26.7 Hz), 31.71 (CH_2), 28.50 (3 × CH_3), 12.56 (CH_3); ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −73.98 (d, 3F, J = 8.5 Hz); GC−MS (CI, CH_4): 272 (100, $[M + 1]^+$), 172 (26), 57 (11); CI-HRMS m/z 272.1466, calcd for $C_{11}H_{21}NO_3F_3$ 272.1473.

(2*S*,4*R*)-, (2*R*,4*S*)-*N*-Ac-5,5,5-trifluoroleucine (7a).[1e] A mixture of (2*S*,4*R*)-, (2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucinol (330 mg, 1.23 mmol), PDC (4.62 g, 12.3 mmol), and dry DMF (2.5 mL) was stirred at room temperature under argon for 4 h and then diluted with 50 mL of ethyl acetate and 50 mL of water. The organic layer was washed with 30 mL of 1 N HCl and 2 × 30 mL of water, dried over $MgSO_4$, and concentrated to give 198 mg of (2*S*,4*R*)-, (2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucine as a pale-brownish oil (60%).

A solution of the above product (180 mg, 0.63 mmol) in 2 mL of CH_2Cl_2 was treated with 0.5 mL of trifluoroacetic acid for 30 min at room temperature. After removal of the solvent, the yellowish residue was dissolved in 2 mL of water and treated with NaOH (126 mg, 3.15) at 0 °C, and acetic anhydride (0.12 mL, 1.26 mmol) was then added dropwise. The reaction mixture was stirred at 0 °C for 30 min and then allowed to warm to room temperature. After stirring for another hour, the mixture was diluted with 30 mL of water, acidified to pH 2 with 3 N HCl, and extracted with ethyl acetate (2 × 90 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to yield 136 mg of **7a** as a white solid (95%). ^{1}H NMR (300 MHz, D_2O) δ 4.48 (dd, 1H, J = 6.1, 8.8 Hz), 2.51 (m, 1H), 2.27 (m, 1H), 2.06 (s, 3H), 1.79 (m, 1H), 1.18 (d, 3H, J = 7.0 Hz); ^{13}C NMR (75.5 MHz, D_2O) δ 175.48 (C=O), 174.60 (C=O), 128.53 (q, CF_3, $^1J_{CF}$ = 278.9 Hz), 51.24 (CH), 34.88 (q, CH, $^2J_{CF}$ = 26.6 Hz), 31.21 (CH_2), 21.90 (CH_3), 13.03 (CH_3); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −73.68 (d, 3F, J = 9.0 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3343s, 3063−2487m (br), 2932m, 2894m, 1709s, 1613s, 1549s, 1266s, 1179s, 1137s; GC−MS (CI, CH_4): 228 (100, $[M + 1]^+$), 211 (47), 186 (26), 140 (16), 57 (11).

(2*S*,4*S*)-, (2*R*,4*R*)-*N*-Ac-5,5,5-trifluoroleucine (7b):[1e] ^{1}H NMR (300 MHz, D_2O) δ 4.48 (dd, 1H, J = 3.8, 11.6 Hz), 2.41 (m, 1H), 2.07 (s, 3H), 2.15−1.91 (br m, 2H), 1.16 (d, 3H, J = 6.9 Hz); ^{13}C NMR (75.5 MHz, D_2O) δ 178.35 (C=O), 177.38 (C=O), 131.09 (q, CF_3, $^1J_{CF}$ = 278.3 Hz), 52.72 (CH), 37.31 (q, CH, $^2J_{CF}$ = 26.6 Hz), 33.06 (CH_2), 24.50 (CH_3), 13.90 (CH_3); ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −73.87 (d, 3F, J = 8.5 Hz); FT-IR (KBr pellet, $\tilde{\nu}_{max}$, cm^{-1}) 3336s, 2977m, 2949m, 2897m, 2615m, 2473s, 1711s, 1628s, 1551s, 1276s, 1250s, 1127s, 1095s; GC−MS (CI, CH_4): 228 (100, $[M + 1]^+$), 211 (47), 186 (26), 140 (16), 120 (3), 57 (11).

(2*S*,4*R*)-5,5,5-Trifluoroleucine (8a).[14] To a solution of **7a** (136 mg, 0.6 mmol) in 2 mL of pH 7.9 aqueous LiOH/HOAc was added porcine kidney acylase I (18 mg) at 27 °C. The mixture was stirred at 27 °C for 48 h (pH was maintained at 7.5 by periodic addition of 1 N LiOH). It was further diluted with 5 mL of water, acidified to pH 5.0, heated to 60 °C with Norit, and filtered. The filtrate was acidified to pH 1.5 and extracted with ethyl acetate (2 × 50 mL). The aqueous layer was freeze-dried to give 63 mg of **8a** (95%). The combined organic layers were concentrated, and the residue refluxed in 3 N HCl for 6 h and was then freeze-dried to yield 64 mg of **8c** (96%).

The other two diastereomers **8b** and **d** were obtained from **7b** using the same procedure as above.

(14) Some data on **8a** and **b** has been reported earlier: See ref 10d.

(2*S*,4*R*)-5,5,5-Trifluoroleucine (8a):[14] ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −74.33 (d, 3F, J = 9.0 Hz); $[\alpha]^{22.9}_D$ +21.6° (*c* 0.5, 1 N HCl).

(2*S*,4*S*)-5,5,5-Trifluoroleucine (8b):[14] ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −74.11 (d, 3F, J = 8.2 Hz); $[\alpha]^{23.6}_D$ −4.0° (*c* 0.8, 1 N HCl).

(2*R*,4*S*)-5,5,5-Trifluoroleucine (8c): ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −74.33 (d, 3F, J = 9.0 Hz).

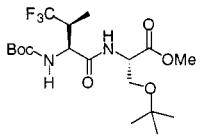
(2*R*,4*R*)-5,5,5-Trifluoroleucine (8d): ^{19}F NMR (282.6 MHz, D_2O/CF_3CO_2H) δ −74.11 (d, 3F, J = 8.2 Hz).

Dipeptides. Boc-TFV-(2*S*,3*S*)-Ser(O*t*-Bu)-OMe(2*S*). To a stirred solution of (2*S*,4*S*)-5,5,5-trifluorovaline (**4b**) (5 mg, 0.02 mmol) in DMF (1 mL) was added diisopropylethylamine (DIEA, 0.01 mL, 0.06 mmol), *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU, 8 mg, 0.02 mmol), and the HCl salt of (2*S*)-*H*-Ser(O*t*-Bu)-OMe (9 mg, 0.04 mmol), sequentially. The mixture was stirred at room temperature for 20 min before dilution with water (5 mL) and extraction with diethyl ether (15 mL). The organic layer was washed with 1 N HCl (2 × 5 mL) and 5% $NaHCO_3$ (2 × 8 mL), dried over $MgSO_4$, and concentrated to give 7 mg of the dipeptide (88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (d, 1H, J = 7.8 Hz), 5.16 (d, 1H, J = 8.7 Hz), 4.65 (m, 1H), 4.39 (dd, 1H, J = 5.1, 8.8 Hz), 3.81 (dd, 1H, J = 2.7, 9.0 Hz), 3.74 (s, 3H), 3.56 (dd, 1H, J = 3.0, 9.0 Hz), 3.04 (m, 1H), 1.46 (s, 9H), 1.23 (d, 3H, J = 7.2 Hz), 1.14 (s, 9H);^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −68.57 (d, 3F, J = 8.7 Hz); HRMS (FAB) *m*/*z* 429.2223, calcd for $C_{18}H_{32}O_6N_2F_3$ 429.2212.

Boc-TFV-(2*S*,3*R*)-Ser(O*t*-Bu)-OMe(2*S*): ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −71.36 (d, 3F, J = 7.9 Hz); HRMS (FAB) *m*/*z* 429.2211, calcd for $C_{18}H_{32}O_6N_2F_3$ 429.2212.

Boc-TFV-(2*R*,3*S*)-Ser(O*t*-Bu)-OMe(2*S*): ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −71.48 (d, 3F, J = 8.5 Hz); HRMS (FAB) *m*/*z* 429.2222, calcd for $C_{18}H_{32}O_6N_2F_3$ 429.2212.

Boc-TFV-(2*R*,3*R*)-Ser(O*t*-Bu)-OMe(2*S*): ^{19}F NMR (282.6 MHz, $CDCl_3$/$CFCl_3$) δ −68.49 (d, 3F, J = 9.0 Hz); HRMS (FAB) *m*/*z* 429.2217, calcd for $C_{18}H_{32}O_6N_2F_3$ 429.2212.



Boc-TFV(2*S*,3*S*)-Ser(O*t*-Bu)-OMe(2*S*)

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Supporting Information Available: NMR spectra (^{1}H, ^{13}C, or ^{19}F) for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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