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Supporting Information

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Biosynthesis and stability of coiled-coil peptides containing (2*S*,4*R*)-5,5,5-trifluoroleucine and (2*S*,4*S*)-5,5,5-trifluoroleucine

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General Procedures

Flash column chromatography was performed on Kiesegel 60 silica gel (230-240 mesh, EM Science). Analytical thin-layer chromatography was performed using E. Merck silica gel Kiesegel 60 F_{254} (0.24 mm) plates. Compounds were made visible by staining with a ninhydrin solution followed by heating. Mass spectra were obtained on a Thermo Finnigan LTQ ESI-MS. Solution of compounds in MeOH:H_2O:AcOH (9:0.9:0.1) were introduced into the instrument by direct infusion. Nuclear magnetic resonance spectra were recorded on a Bruker DPX-300 instrument using standard deuterated solvents.

Stereochemical Resolution of 3 and 4 from 2

The racemic mixture of *N*-Boc *t*-butyl esters of trifluoroleucine (TFL) could be easily separated into the two enantiomeric pairs **5a** and **5b** by flash column chromatography. In addition, racemization at C2 allowed for recycling of the undesired 2*R* diastereomers (Scheme S1). Assignment of absolute stereochemistry and corresponding ^{1}H NMR spectra have been previously reported for compounds **3** and **4**.[1] Analytical data for new compounds are reported below. Procedures for all other compounds were adapted from literature.[1]

***N*-Boc-5,5,5-TFL *t*-butyl esters** (**5a** and **5b**)

TFL (**2)** was converted to the *N*-Boc derivative using literature procedures.[2] *N*-Boc TFL (2.05 g, 7.03 mmol) was added to anhydrous *t*-BuOH (100 mL) maintained at 32 °C. To this solution, $(Boc)_2O$ (1.73 g, 7.93 mmol) and DMAP (88 mg, 0.72 mmol) were added in that order. The reaction was stirred under argon for 1 h. The product was purified by flash chromatography using *n*-pentane/ methyl *t*-butyl ether (MTBE) (20:1) as the eluent to give 560 mg of **5a** as a white solid and 561 mg of **5b** as a pale yellow oil. A mixture of diastereomers (162 mg) was also recovered, resulting in an overall yield of 52% from **2**.

(2*S*,4*S*)-,(2*R*,4*R*)-*N*-Boc-5,5,5-trifluoroleucine *t*-butyl ester (5b)

R_f: pentane/MTBE (5:1) 0.43; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20-1.22 (d, 3H, J = 6.9 Hz), 1.45 (s, 9H), 1.47 (s, 9H), 1.76-1.84 (dt, 2H, J = 3.9, 9.9 Hz), 2.2-2.4 (m, 1H), 4.24-4.26 (m, 1H), 4.94-4.97 (d, 1H, J = 8.1 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.9, 156.1, 130.4, 126.7, 82.89, 80.51, 51.72, 35.31 (q, $^2J_{CF}$ = 26.9 Hz), 33.42, 30.13, 28.68, 28.36, 12.70; ESI-MS *m*/*z* 364.20 (100, $[M+23]^+$), calculated for $C_{15}H_{26}NO_4F_3$ 341.37.

(2*S*,4*R*)-,(2*R*,4*S*)-*N*-Boc-5,5,5-trifluoroleucine t-butyl ester (5a)

R_f: pentane/MTBE (5:1) 0.38; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18-1.20 (d, 3H, J = 6.9 Hz), 1.45 (s, 9H), 1.48 (s, 9H), 1.57-1.67 (m, 2H), 2.07-2.16 (m, 1H), 2.37-2.39 (m, 1H) 4.20-4.22 (m, 1H), 5.10-5.14 (d, 1H, J = 6.0 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.5, 155.5, 130.3, 126.6, 83.08, 80.43, 52.43, 35.45 (q, $^2J_{CF}$ = 26.2 Hz), 33.64, 30.12, 28.70, 28.37, 13.36; ESI-MS: *m*/*z* 364.17 (100, $[M+23]^+$), calcd for $C_{15}H_{26}NO_4F_3$ 341.37.

(2*S*,4*R*)-5,5,5-trifluoroleucine (4)

To a solution of **5a** (622 mg, 1.82 mmol**)** in 4 mL CH_2Cl_2 was added CF_3CO_2H (4 mL). The solution was stirred at room temperature for 1 h. After removal of the solvent, the resulting residue was dissolved in 4 mL 1N HCl and extracted with 3 × 5 mL CH_2Cl_2.

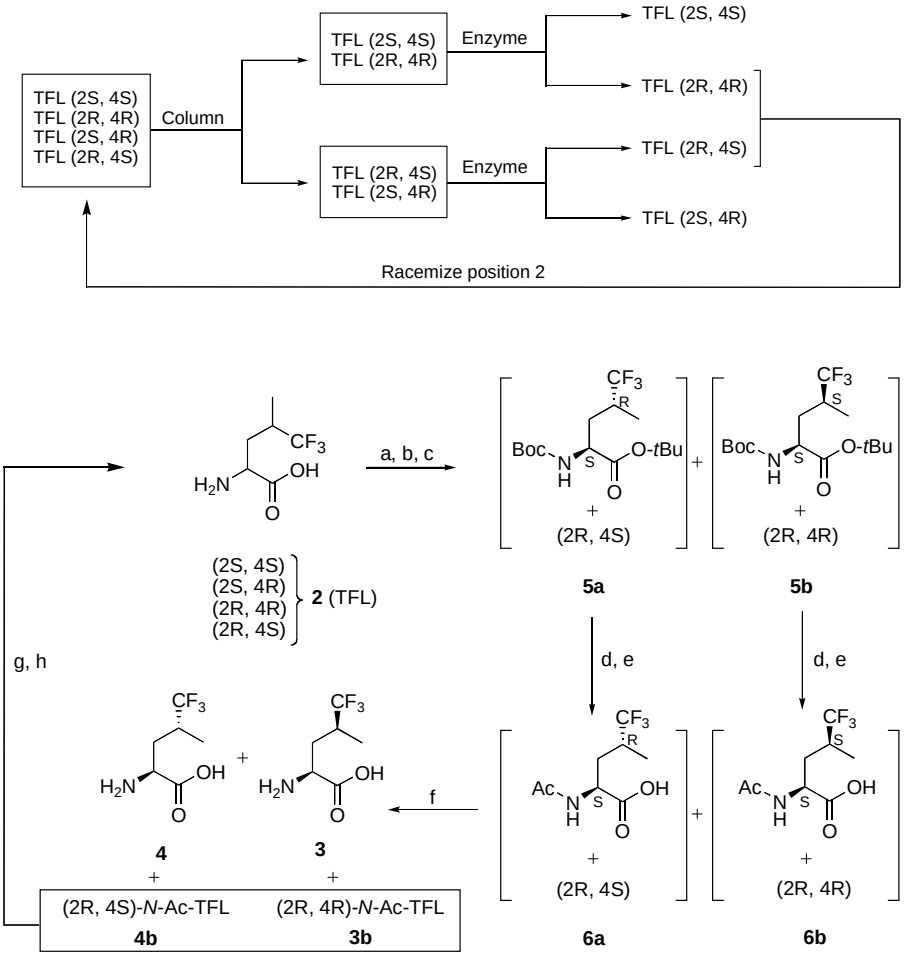
The solvent (water) was removed by rotary evaporation and freeze drying. The corresponding TFL·HCl salt was obtained in 87% yield (352 mg). A 5 mL aqueous solution of TFL·HCl (352 mg, 1.58 mmol) and NaOH (253 mg, 6.32 mmol) was cooled to 0 °C. To this solution, Ac_2O (224 μL, 2.37 mmol) was added dropwise. The mixture was acidified to pH 2.5 with conc. HCl, and extracted with 3 × 100 ml EtOAc. The combined organic layers were dried over $MgSO_4$. Rotary evaporation of the solvent yielded **6a** as a white solid (314 mg, 88%).

Compound **6a** (314 mg, 1.38 mmol) was subjected to enzymatic resolution as previously described. Compound **4b** was recovered from the reaction (190 mg). Compound **4** was obtained in 59% yield (71 mg).

Compound **3** was obtained from **6b** using the same procedures as above.

Deacetylation and Racemization of (2*R*,4*R*)-*N*-Acetyl-5,5,5-trifluoroleucine (3b)[3]
Compound **3b** (113 mg, 0.49 mmol) was refluxed in 3N HCl for 3 hours. The solvent was removed by rotary evaporation and lyophilization. The residue so obtained was dissolved in 25 mL of acetic acid to which 0.05 eq of benzaldehyde was added. The reaction was stirred at 90 °C for 12 h. The solvent was removed by rotary evaporation. The residue was dissolved in 25 mL 1N HCl, then extracted with 3 × 20 mL EtOAc. Removal of water delivered **2** in quantitative yield, and with C2 judged to be racemic by ^{1}H NMR.

Scheme S1



(a) Boc_2O, $NaHCO_3$, CH_3OH, sonicate, 1 h; (b) Boc_2O, DMAP, *t*BuOH (anhyd); (c) flash column chromatography, *n*-pentane/MTBE (20:1); 52% over 3 steps from **2**; (d) CF_3CO_2H:CH_2Cl_2 (1:1); (e) NaOH/H_2O, Ac_2O, 0 °C; (f) Porcine kidney acylase I, pH 7.5, 25 °C; 3N HCl; 54% over 3 steps from **5a** or **5b**; (g) 3N HCl, reflux; (h) CH_3CO_2H, 0.1 eq. C_6H_5CHO, 90 °C, 6 h; 95% over 2 steps from (**3b** + **4b**).

Table 1S. Melting Transitions from CD

Protein	T_m (°C)
Leu-A1	55
SS-A1	65
SR-A1	65
SS-A1•SR-A1[a]	68

[a] This sample is an equimolar mixture of SS-A1 and SR-A1. A1 made from the SS-SR isomer mixture **2** exhibited a T_m of 67°C.[4]

1

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