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Enantioselective synthesis 5-fluoro-L-DOPA *via* chemoenzymatic route

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Material and methods

The ^{1}H NMR spectra were measured with Bruker Avance 300 instruments in $CDCl_3$ at 30°C. Mass spectra (EI, 70eV, direct injection) were recorded with a Finnigan SSQ-7000 instrument. Optical rotations were measured with a Perkin-Elmer (model 341) polarimeter in 0.5 dm cells at 25 °C. Melting points were measured on an Electrothermal IA 9000 series device in sealed capillaries. Chiral column - Chirobitic TAG column 250×4.6 mm; eluent: MeOH / H_2O (60 / 40) + 0.2% DEA; 0.70 ml min^{-1}, UV-detector 210 nm). 2-Fluorophenol (Aldrich), pyridoxal-5′-phosphate (PLP) (USB Corp.) and all other reagents were used without further purification. Enzyme experiments were performed using distilled deionized water.

3-Fluoro-L-tyrosine **3**

A mixture of potassium pyruvate (1.6 g), ammonium acetate (24 g), 2-fluorophenol **2** (3.2 g) was loaded into a flask with *Citrobacter freundii* cells (2.4 g) in phosphate buffer (pH 8, 1.6 dm^3). Pyridoxal phosphate solution (1.5 ml) was added (C = 4.10^{-4} mol), and an ammonia solution until pH 8.2 reached. Freshly grown cells of the bacteria *Citrobacter freundii* were obtained in a nutrient medium containing, in terms of liter, enzymatic casein hydrolyzate (10 g), acid casein hydrolyzate (2 g), yeast extract (5 g), L-tyrosine (1 g) and pyridoxine hydrochloride (0.05 g). The solution was shaken at 30°C for 12 h. The reaction was monitored by TLC in amino acid buffer (3-fluorotyrosine R_f = 0.2), visualization with ninhydrin. The reaction mixture was centrifuged to separate the cells, the solution was evaporated to 200 ml. The white precipitate of product **3** was separated by filtration. The solution was applied to a DOWEX 50W (H^+) column, eluted with 5% NH_3 aq., and evaporated to give additional product. Total yield was 5.45 g (96%), m p. 260°C. $[\alpha]_D$ − 5.9° (HCl, 20°C). Lit.[S1] m. p. 260-261°C, $[\alpha]_D$ − 5.7° (HCl, 26 °C). ^{1}H NMR (D_2O): 6.84–6.97 (m, 3H, Aryl) 3.81 (m, 1H, CH), 3.95 (m, 1H, CH_2), 2.90 (m, 1H, CH_2). ^{13}C NMR (D_2O): 173.8 (COOH) 150.5, 142.9, 127.4 125.6 118.2, 116.85, 55.9, 35.4. ^{19}F NMR: −136.8. Found, %: C (54.39), H (5.28), N (6.98). $C_9H_{10}NO_3F$. Calculated, %: C (54.27), H (5.06), N (7.03).

3-Nitro-5-fluoro-L-tyrosine **4**

A suspension of 3-fluorotyrosine **3** (10 g) in water (40 ml) was cooled to 0°C and treated slowly with stirring with conc. HNO_3 (27 ml). The solution was heated to 25°C, yellow crystals filtered off, dissolved in water at 80°C, added NH_3 aq. to pH 5. The precipitate was filtered off, washed with cold water, and recrystallized from aqueous ethanol (discolored with activated charcoal). Yield of **4** was 11.2 g (91%). ^{1}H NMR (D_2O): 7.16, 7.35 (2d, 2H, Ar), 4.05 (m, 1H, Ar -R-CH), 3.20 (m, 1H, Ar-CH), 3.15 (m, 1H, Ar-CH); *ee* >97% (HPLC). Lit.[S2]: ^{1}H NMR (D_2O): 7.31, 7.67 (2d, 2H, Ar), 4.16 (m, 1H, Ar -R-CH), 3.16 (m, 1H, Ar-CH), 3.03 (m, 1H, Ar- CH). Found, %: C (44.53), H (3.92), N (11.19). $C_9H_9N_2O_5F$. Calculated,%: C (44.27), H (3.71), N 11.47).

3-Amino-5-fluoro-L-tyrosine **5**

To 3-nitro-5-fluorotyrosine **4** (10.0 g) in water (35 ml) was added HCl conc. (40 ml), and this was boiled with tin dust (18.0 g). After hydrogen evolution ceased, the solution was filtered off and evaporated to dryness in a vacuum. The residue was dissolved in water, H_2S was passed through the solution until SnS completely precipitated, and filtered. The solution was boiled, evaporated in vacuum to a small volume, 2 N KOH added to pH ~ 6.5 and cooled. The precipitated crystals were washed with water at 0°C and recrystallized from water in the presence of activated charcoal. Yield of 3-amino-5-fluoro-L-tyrosine **5**: 8.4 g (88%). $[\alpha]^{15}_D = -3.61$ (C1, 4N HCl). ^{1}H NMR (D_2O): 6.46 (m, 2H, C_6H_2), 4.09 (m, 1H, CH), 3.03 (m, 1H, CH_2), 2.86 (m, 1H, CH_2); ^{13}C NMR (D_2O): 171.7 (COOH), 152.7, 150.7, 138.3, 126.7 120.2, 117.7, 54.3, 34.7. ^{19}F NMR: −133.26. *ee* >96% (HPLC). Found, %: C (50.13), H (5.42), N (12.92). $C_9H_{11}N_2O_3F$. Calculated,%: C (50.47), H (5.18), N (13.08).

5-Fluoro-L-3,4-dihydroxyphenylalanine **1**

A solution of $CuSO_4 \cdot 5H_2O$ (2.85 g, 0.0114 mol) in water (12 ml) was heated to boiling in a three-necked flask with a condenser in a stream of N_2. Separately, $Ba(NO_3)_2 \cdot H_2O$ (0.234 g, 0.00095 mol) was dissolved in water (3 ml) and added dropwise to a mixture of 3-amino-5-fluorotyrosine **5** (0.40 g, 0.0019 mol) in cold 17% H_2SO_4 (3.2 ml, 0.0042 mol) at 0°C. $BaSO_4$ precipitated, and the mixture was poured without filtration into a boiling $CuSO_4$ solution. The dark red solution was cooled, the precipitate was filtered off, H_2S was passed through the filtrate for 3 hours, CuS was filtered on a Schott filter and a yellow solution (pH ~ 1) was obtained. The solution was adjusted to pH 5 by adding $Ba(OH)_2$ solution, concentrated *in vacuo* and cooled at +5°C. The precipitated brown crystals were filtered off. Crystals were additionally isolated from the solution by evaporation, extraction with ethanol and combined. Yield of target product **1**: 0.27 g (68%). $[\alpha]_D^{20}$ = + 2.4 (*c* 0.5, 1 N HCl). (Lit.[S3]; $[\alpha]_D^{20}$ = + 2.5 (*c* 0.32, 1 M HCl)). Chiral HPLC (for analysis details, see Ref. S4): *ee* > 96%. ^{1}H NMR (D_2O): 6.57 (m, 2H, Aryl) 3.85 (m, 1H, CH), 2.95 and 3.04 (1H, m, CH_2). ^{13}C NMR (D_2O): 173.9 (COOH), 153.4, 151,0 135.2, 127.5 114.3, 108.9, 55.9, 35.8. ^{19}F NMR: −135.7. Found, %: C (50.03), H (4.82), N (6.42). $C_9H_{10}NO_4F$. Calculated,%: C (50.24), H (4.68), N (6.51).

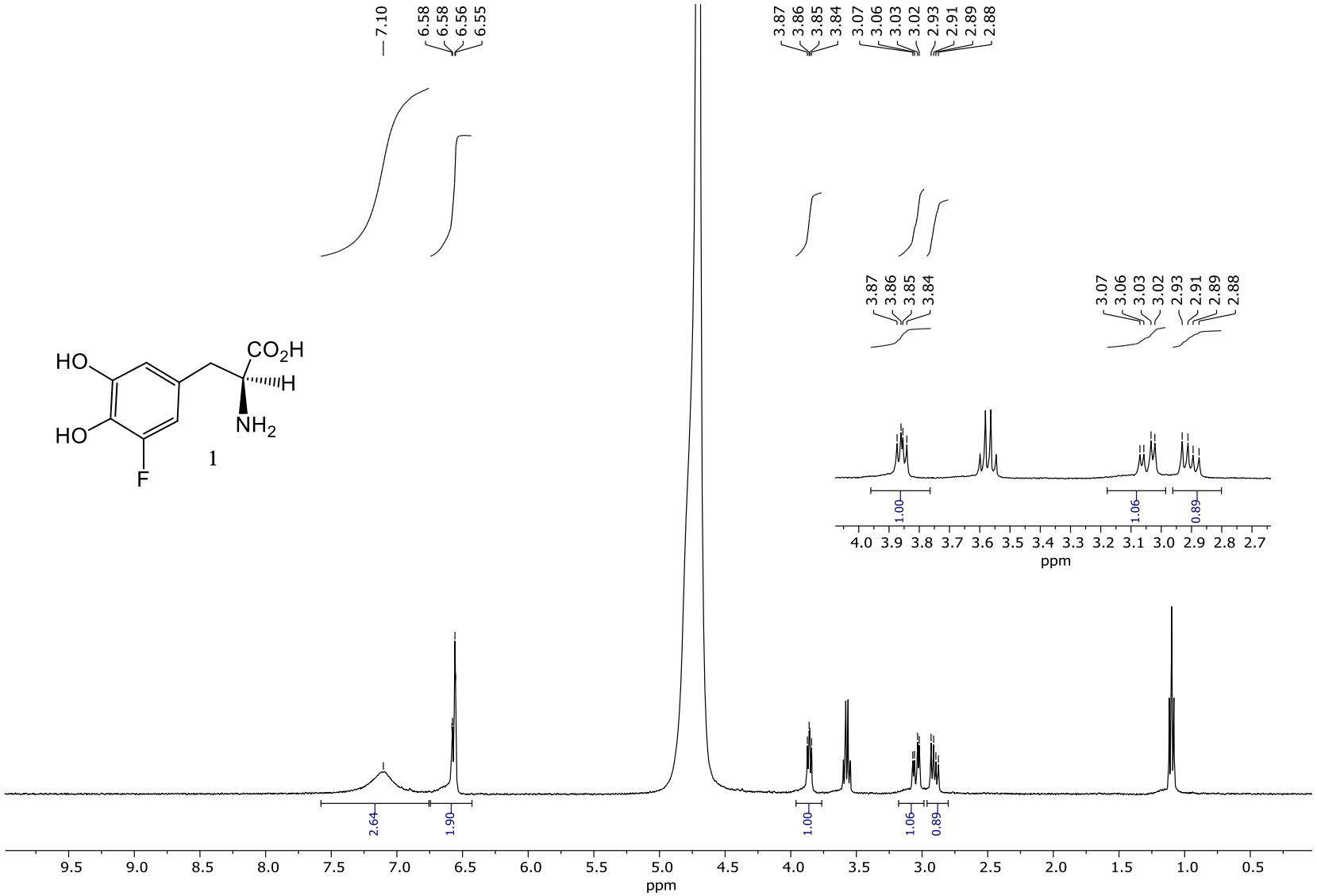

Figure S1. ^{1}H NMR spectrum of F-DOPA **1**.

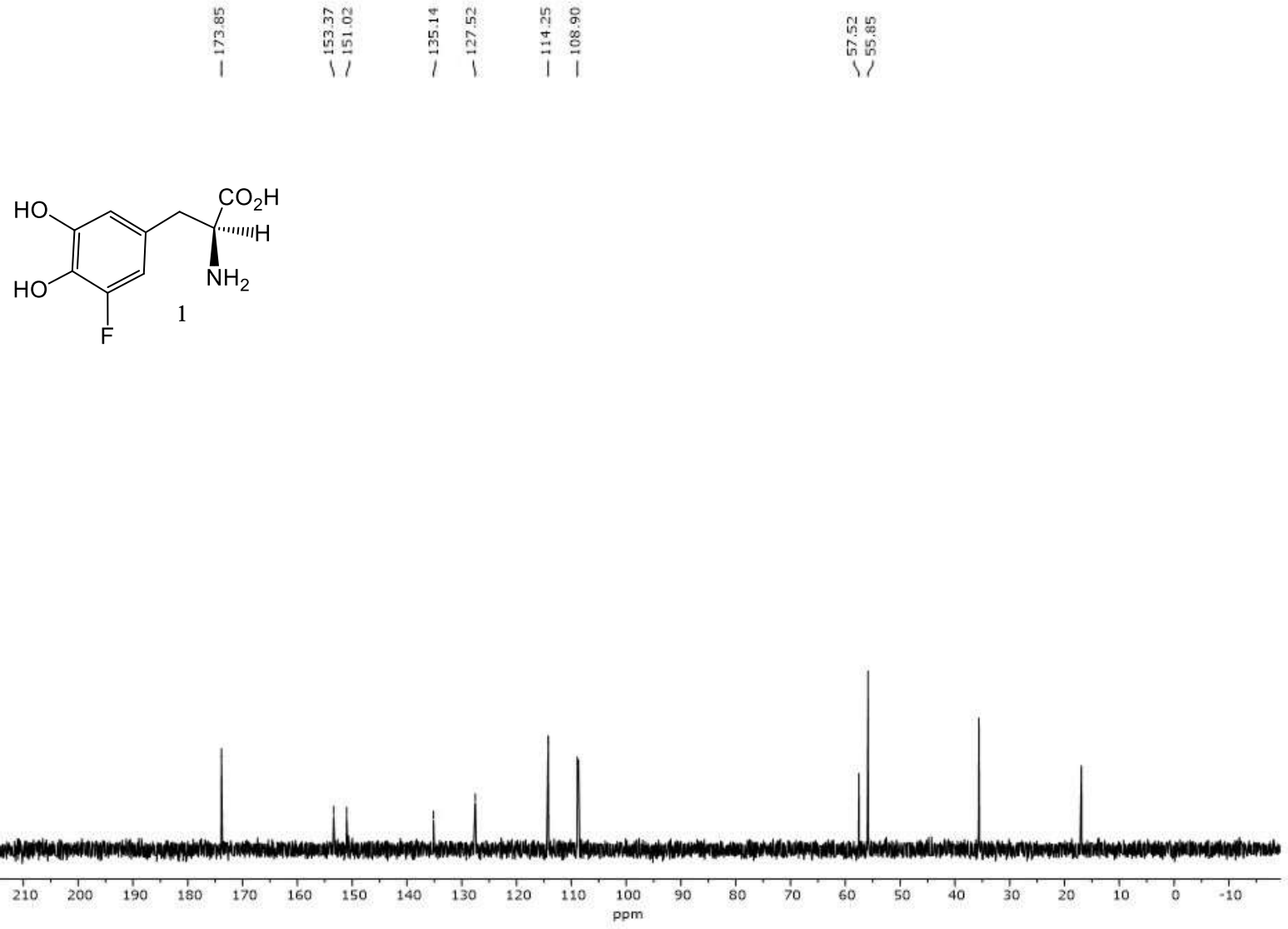

Figure S2 ^{13}C NMR spectrum of F-DOPA **1**.

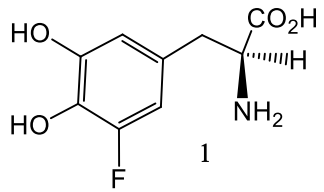

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Figure S3 ^{19}F NMR spectrum of F-DOPA **1**.

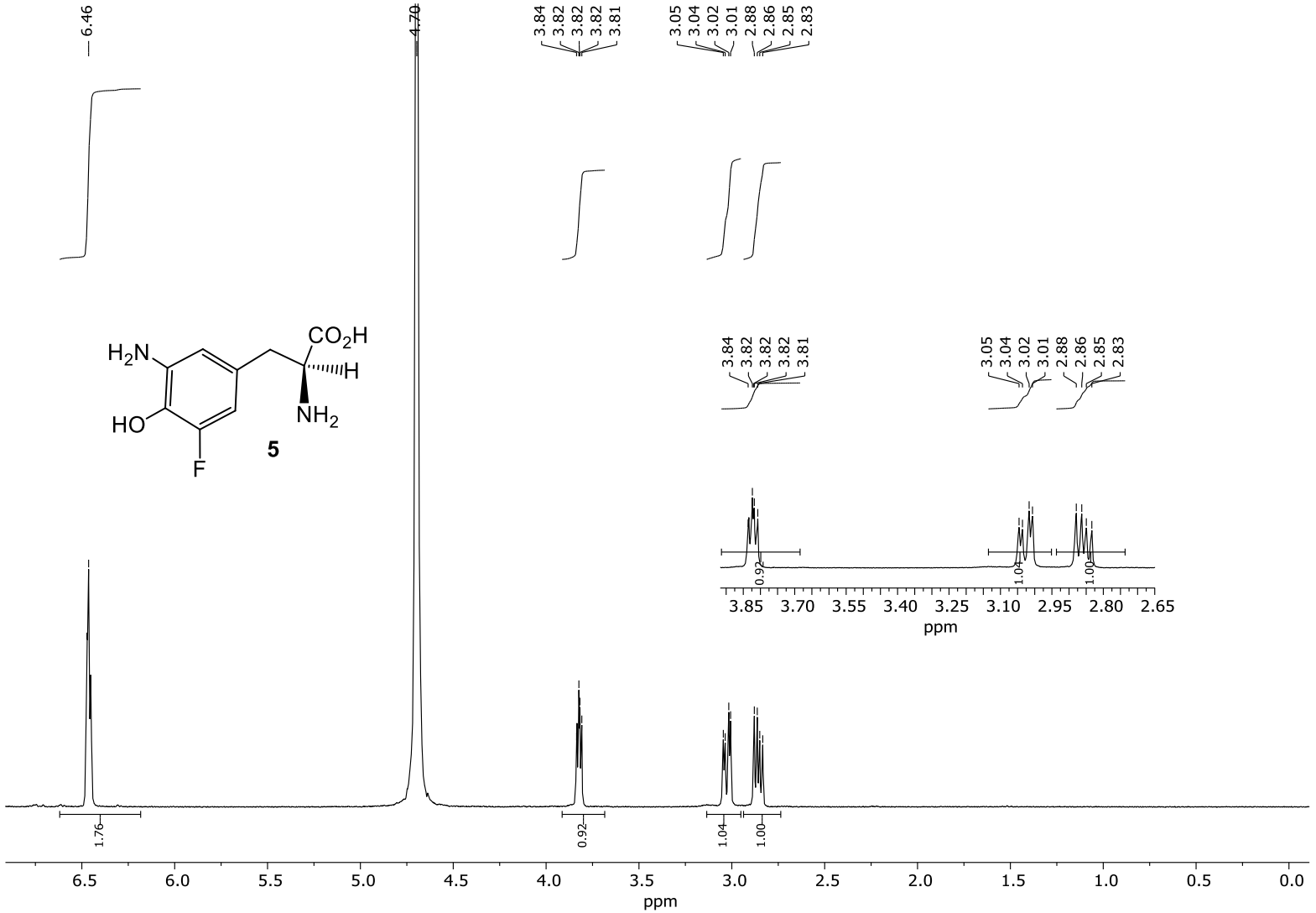

Figure S4 ^{1}H NMR spectrum of 3-amino-5-fluoro-L-tyrosine **5**.

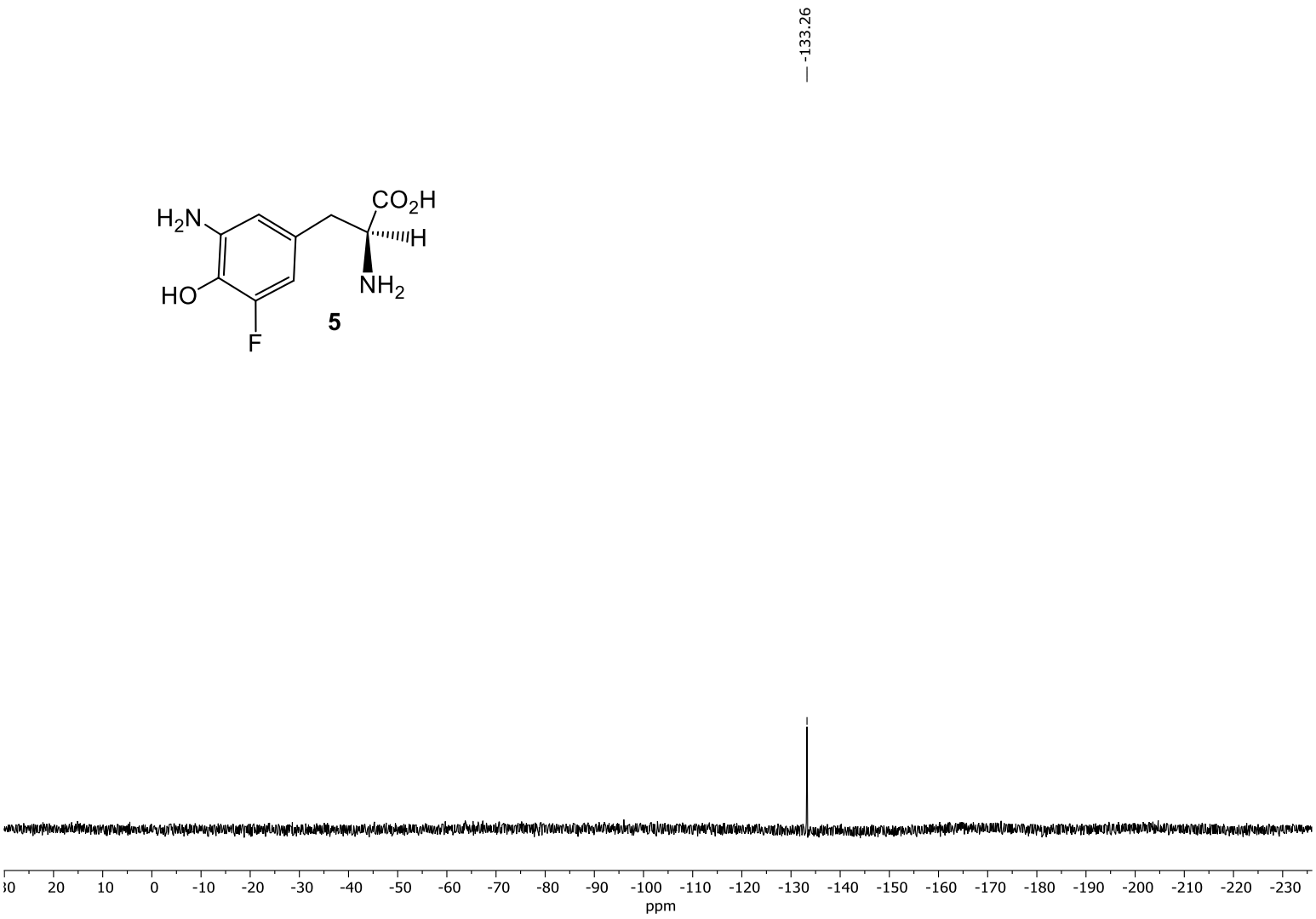

Figure S5 ^{19}F NMR spectrum of 3-amino-5-fluoro-L-tyrosine **5**.

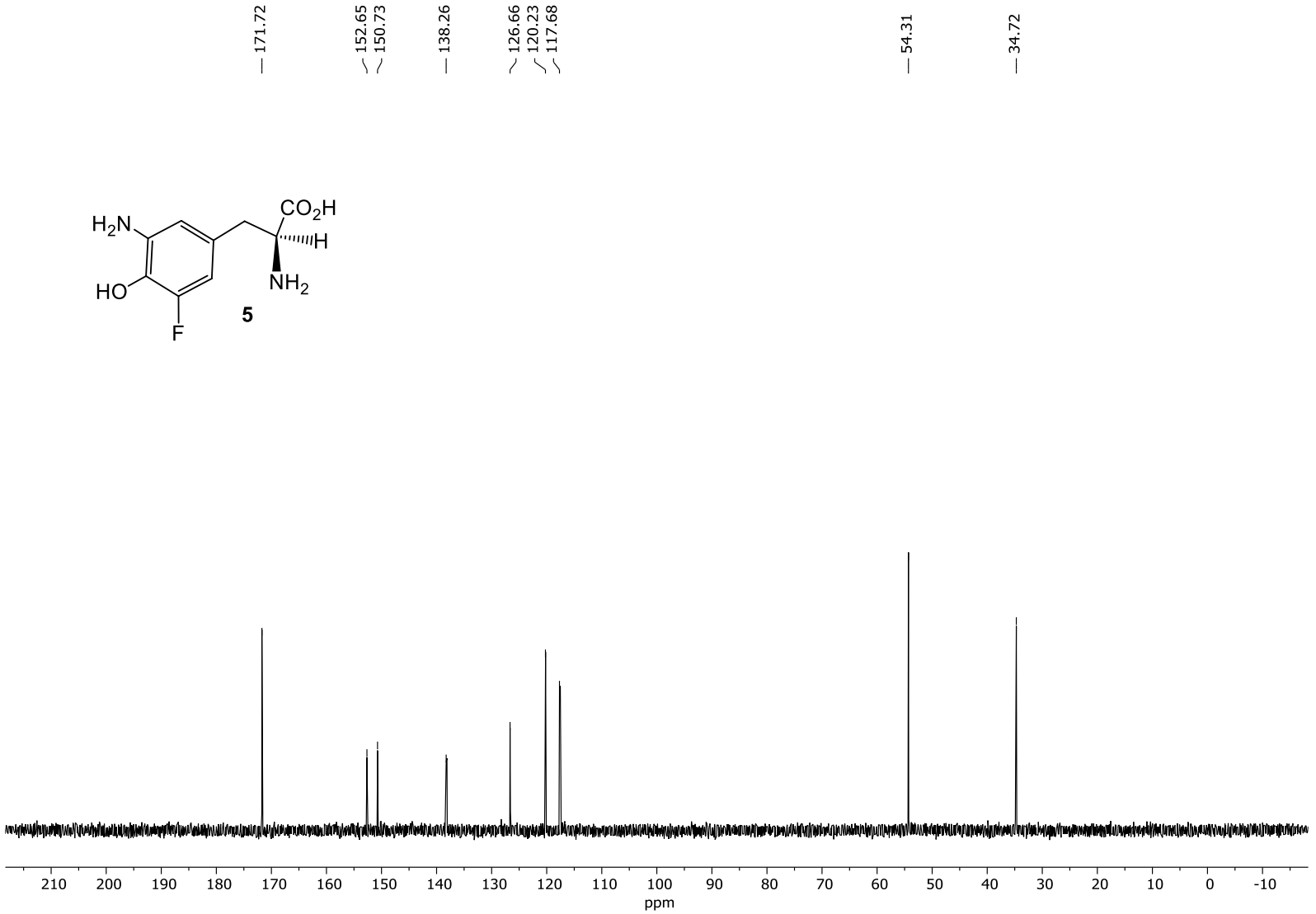

Figure S6 ^{13}C NMR spectrum of 3-amino-5-fluoro-L-tyrosine **5**.

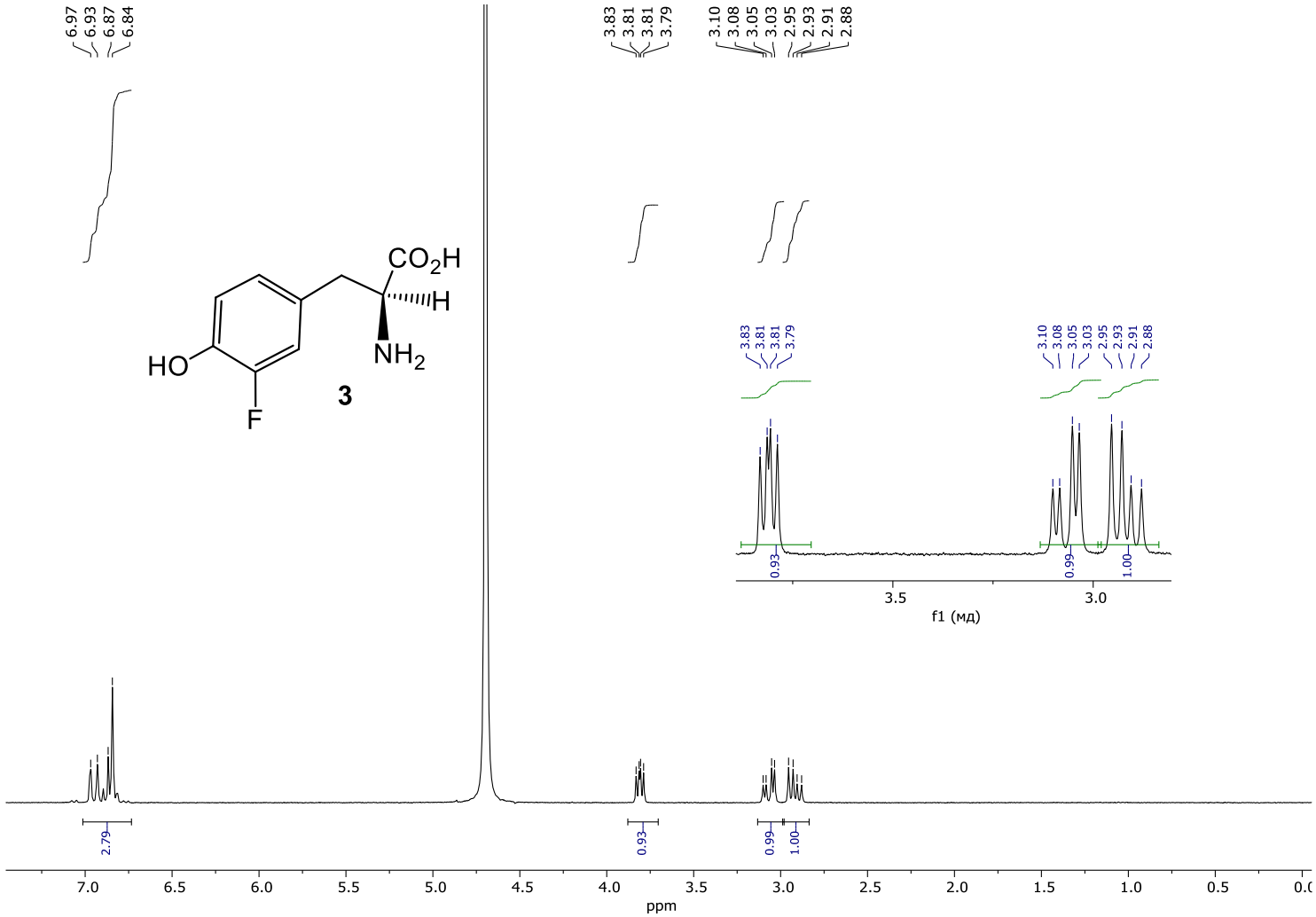

Figure S7 ^{1}H NMR spectrum of 3-fluoro-L-tyrosine **3**

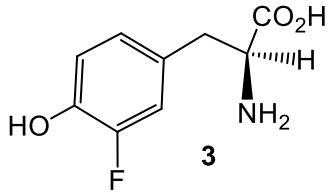

Figure S8 ^{19}F NMR spectrum of 3-fluoro-L-tyrosine **3**

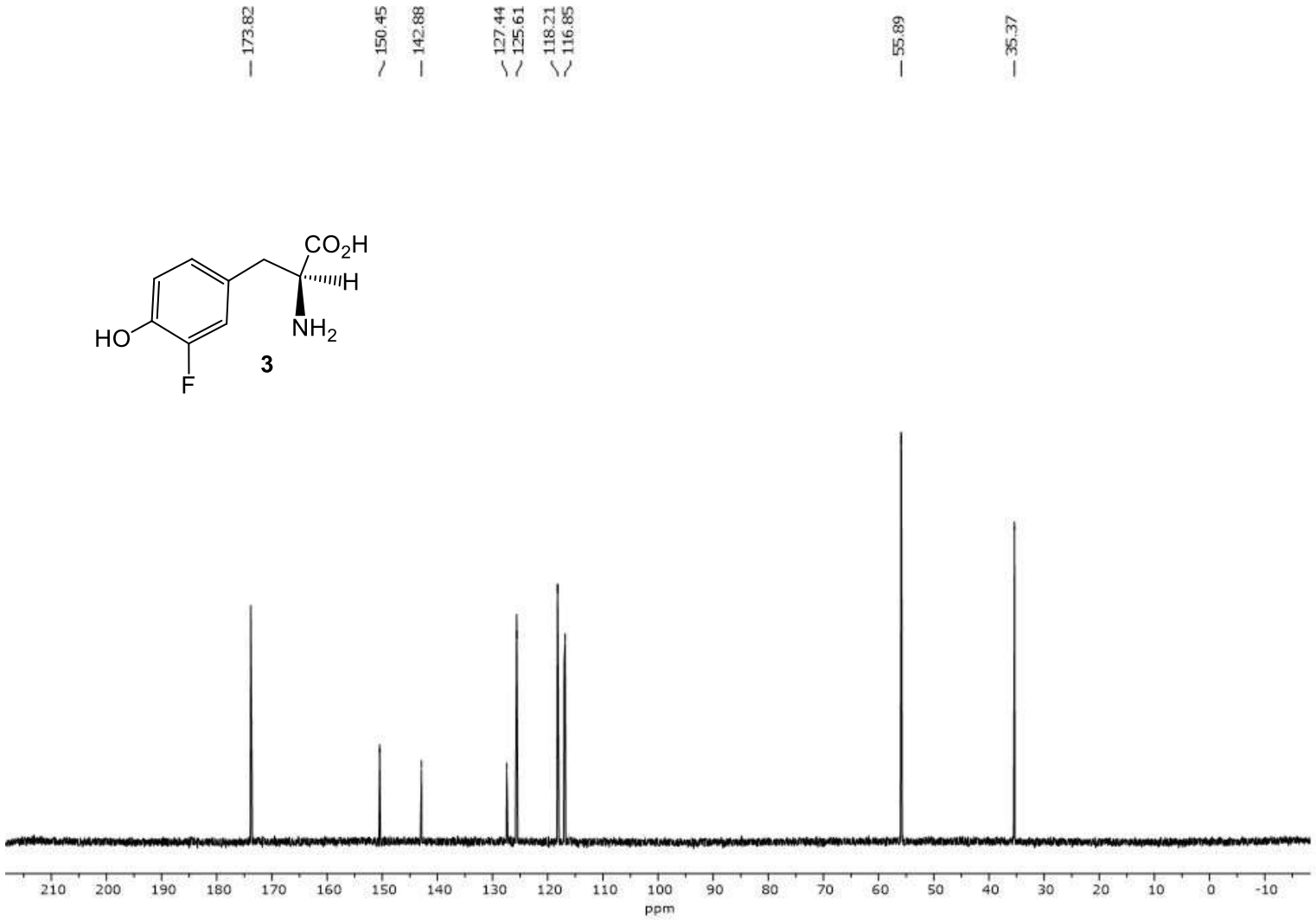

Figure S9 ^{13}C NMR spectrum of 3-fluoro-L-tyrosine **3**

References:

S1**.** C. Niemann and M. M. Rapport, *J. Am. Chem. Soc*., 1946, **68**, 1671.

S2. N. Vasdev, R. Chirakal, R. Ashique, G. J. Schrobilgen and K. Gulenchyn, *J. Fluorine Chem*., 2003, **121**, 9.

S3. R. Chirakal, N. Vasdev, G. J. Schrobilgen and C. Nahmias, *J. Fluorine Chem*., 1999, **99**, 87.

S4. S. Kaneko, K. Ishiwata, K. Hatano, H. Omura, K. Ito and M. Senda, *Appl. Radiat. Isotopes*, 1999, **50**, 1025.