N^1-Fluoroalkyltryptophan analogues: synthesis and *in vitro* study as potential substrates for indoleamine 2,3-dioxygenase

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Supporting Information

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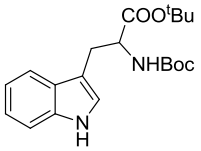
1. Organic Syntheses

1. Materials and methods.

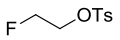
All chemicals and reagents purchased from Sigma Aldrich, Merck, Iris Biotech or VWR were of analytical grade and used without further purification. All solvents used were HPLC grade. HPLC analyses were run on a Waters system (600 pump, 996 PDA UV detector (200 – 400 nm) controlled by the Empower software. Analytical HPLC analyses were performed on X-Terra® RP18 (150 x 4.6 mm, 3.5 µm) column from Waters, with CH_3CN and H_2O containing 0.1% TFA (proportions given in parentheses) at 0.7 mL/min. Semi-preparative HPLC analyses were performed on X-Terra® Prep RP18 (10 x 250 mm, 10 µm) column from Waters at 4 mL/min. Chiral HPLC analyses were performed on Astec Chirobiotic™ T (CH_3OH/H_2O + 0.1% TFA 85/15 (v/v), 0.8 mL/min). UPLC analyses were run on a Waters system (Acquity UPLC® PDA UV eλ detector (190 – 400 nm) controlled by the Empower software) and were performed on ACQUITY UPLC® BEH C18 (2.1 x 100 mm, 1.7 µm) column from Waters (solvents and proportions given in parentheses), at 0.7 mL/min and 50 °C. TLC analyses were performed on silica gel Polygram® SIL G/UV_{254} pre-coated TLC-sheets. ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on Bruker 250 MHz (and 400 MHz for ^{19}F NMR analyses). Chemical shifts δ are given in ppm. The employed abbreviations for the multiplicities are the following ones: s = singlet, d = doublet, t = triplet, q = quadruplet, p = quintet, m = multiplet, br = broad. The coupling constant *J* is given in Hz. Spectra were recorded as solutions in d_1-$CDCl_3$ (d_H at 7.26 ppm, d_C at 77.16 ppm) or d_2-D_2O (d_H at 4.79 ppm) which were used as internal references. The melting point of solid compounds was determined on Melting Point (Mp) Instrument Digital 9000 Series IA9100 230V. Mass spectra (MS) were recorded on Finnigan Thermoquest TSQ7000 mass spectrometer (ThermoElectronCorp.) operating in full-scan MS mode with an electrospray source (ESI+/-). FT-MS mass analyses were performed on an ESI-FT-ICR mass spectrometer (SolariX, Bruker) in positive ion mode. Extern calibration was done over the range of m/z 50 to 1500 using H_3PO_4 adducts and mean residual error obtained was < 0,5 ppm. Internal correction was applied using GluFib peptide standard (Waters). Samples were analyzed at 13 µM in H_2O/CH_3CN (50/50), formic acid 0.1% solution supplemented with 0,33 µM GluFib.

2. Synthesis of *tert*-butoxycarbonyl-L-tryptophan *tert*-butyl ester (2).



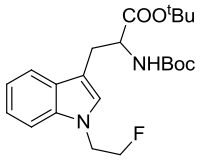
tert-Butoxycarbonyl-L-tryptophan **1** (15.22 g, 50 mmol) was dissolved in dichloromethane (250 mL) and was stirred at 0 °C for 10 minutes. *tert*-Butanol (7 mL, 75 mmol), DCC (12.38 g, 60 mmol) and DMAP (61 mg, 5 mmol) were added successively and the resulting solution mixture was stirred 5h at 0 °C and then 20h at room temperature. After diethylether addition (250 mL), and filtration three times over celite, the solvent was evaporated. The residue was extracted with EtOAc and washed with aq. $KHSO_4$ (5 %wt), aq. $NaHCO_3$ sat., water and brine. The organic layer was concentrated and purified by chromatography on silica gel (CH_2Cl_2/petroleum ether 60-80/EtOAc 9/2/1 (v/v/v)) to give the product as a white crystalline product (4.87 g, 27%). R_f (CH_2Cl_2/petroleum ether 60-80/EtOAc 9/2/1 (v/v/v)): 0.58; R_f (petroleum ether 60-80/EtOAc 4/1 (v/v)): 0.20. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.37 (s, 1H, NH_{indol}), 7.62 (d, *J* = 7.6 Hz, 1H, CH_{arom}), 7.34 (d, *J* = 7.6 Hz, 1H, CH_{arom}), 7.15 (dt, *J* = 18.4, 7.2 Hz, 2H, CH_{arom}), 6.99 (s, 1H, CH_{indol}), 5.11 (d, *J* = 7.8 Hz, 1H, NH_α), 4.56 (dd, *J* = 13.2, 5.8 Hz, 1H, CH_α), 3.40 – 3.11 (m, 2H, CH_2), 1.41 (d, *J* = 12.0 Hz, 18H, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.59 (**C**O), 155.42 (**C**O), 136.20 ($\mathbf{C}_{arom}$), 127.99 ($\mathbf{C}_{arom}$), 122.84 ($\mathbf{C}H_{indol}$), 122.09 ($\mathbf{C}H_{arom}$), 119.48 ($\mathbf{C}H_{arom}$), 119.11 ($\mathbf{C}H_{arom}$), 111.22 ($\mathbf{C}H_{arom}$), 110.53 ($\mathbf{C}_{indol}$), 81.89 ($\mathbf{C}_{tBu}$), 79.71($\mathbf{C}_{tBu}$), 54.86 ($\mathbf{C}H_\alpha$), 28.44 ($\mathbf{C}H_2$), 28.03 ($\mathbf{C}H_3$). MS: (ESI, positive): *m/z* = 361 [M+H], 383 [M+Na]; (ESI, negative): *m/z* = 359 [M-H]. FT-MS (positive): *m/z* = 361.212184 (calculated), 361.212193 (measured) [M+H]. Mp: 185-186 °C (in agreement with corresponding literature[1]).

3. Synthesis of 1-fluoro-2-tosyloxyethane (3).



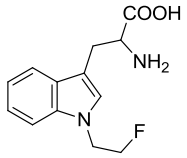
p-Toluenesulfonyl chloride (20.01 g, 105 mmol) in dichloromethane (25 mL) was slowly added to a solution of 2-fluoroethanol (**6**) (6.41 g, 100 mmol) in dichloromethane (80 mL) and triethylamine (28 mL, 200 mmol). The reaction mixture was stirred for 6h at room temperature. Then, a $NaHCO_3$ saturated aqueous solution was added and the aqueous solution was extracted three times with dichloromethane. The organic layers were dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was finally purified by flash chromatography on silica gel (Hexane/EtOAc 4/1 (v/v)) to give pure 1-fluoro-2-tosyloxyethane (20.74 g, 95%) as a colorless oil, which solidified when placed at -20 °C. R_f (Hexane/EtOAc 9/1 (v/v)): 0.21. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.3 Hz, 2H, Ph), 7.33 (d, *J* = 8.2 Hz, 2H, Ph), 4.53 (dt, $^2J_{H-F}$ = 47.2 Hz, and $^3J_{H-H}$ = 5.0 Hz, 2H, C$\mathbf{H_2}$-F), 4.22 (dt, $^3J_{H-F}$ = 27.5 Hz, and $^3J_{H-H}$ = 4.9 Hz, 2H, O-C$\mathbf{H_2}$-CH_2-F), 2.41 (s, 3H, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 145.24 ($\mathbf{C}_{arom}$), 132.51 ($\mathbf{C}_{arom}$), 129.97 (2 $\mathbf{C}H_{arom}$), 127.88 (2 $\mathbf{C}H_{arom}$), 80.59 (d, $^1J_{C-F}$ = 173.2 Hz, $\mathbf{C}H_2$-F), 68.69 (d, $^2J_{C-F}$ = 20.6 Hz, O-$\mathbf{C}H_2$-CH_2-F), 21.57 ($\mathbf{C}H_3$). MS: (ESI, positive): *m/z* = 219 [M+H] and 241 [M+Na] (in agreement with corresponding literature[2]).

4. Synthesis of *tert*-butoxycarbonyl-(1-(2-fluoroethyl)-tryptophan) *tert*-butyl ester (4).



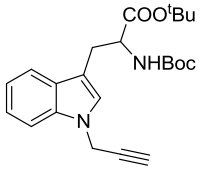
A solution of *tert*-butoxycarbonyl-*L*-tryptophan *tert*-butyl ester **2** (72.5 mg, 0.2 mmol) in 10 mL of DMF was mixed with sodium hydride (20 mg, 0.5 mmol) for 20 minutes at 0 °C and under N_2. A solution of 1-fluoro-2-tosyloxyethane **3** (48 mg, 0.22 mmol) in DMF (2 mL) was added dropwise. This solution was mixed for 2h at 0 °C and then allowed to warm progressively to room temperature. After 24h, the reaction was quenched with NH_4Cl (5 %wt in water). The aqueous solution was extracted 3 times with EtOAc. The organic layers were combined, dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was finally purified by chromatography on silica gel (CH_2Cl_2/Hexanes/EtOAc 9/2/1 (v/v/v)) to give the product (**4**) as a colorless oil (42.5 mg, 52%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.61 (d, *J* = 7.8 Hz, 1H, CH_{arom}), 7.28 (d, *J* = 7.8 Hz, 1H, CH_{arom}), 7.24 – 7.18 (m, 1H, CH_{arom}), 7.15 – 7.09 (m, 1H, CH_{arom}), 6.98 (s, 1H, CH_{indol}), 5.09 (d, *J* = 8.8 Hz, 1H, NH_α), 4.67 (dt, $^2J_{H\text{-}F}$ = 47.02 Hz and $^3J_{H\text{-}H}$ = 4.89 Hz, 2H, C**H$_2$**-F), 4.55 – 4.45 (m, 1H, CH_α), 4.43 (dt, $^3J_{H\text{-}F}$ = 26.04 Hz and $^3J_{H\text{-}H}$ = 4.88 Hz, 2H, C**H$_2$**CH$_2$-F), 3.32 – 3.16 (m, 2H, CH_2), 1.43 (s, 9H, CH_3), 1.38 (s, 9H, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.36 (**C**O), 155.28 (**C**O), 136.26 (**C**$_{arom}$), 128.70 (**C**$_{arom}$), 126.73 (**C**H$_{indol}$), 121.94 (**C**H$_{arom}$), 119.46 (**C**H$_{arom}$), 119.35 (**C**H$_{arom}$), 110.11 (**C**$_{indol}$), 109.02 (**C**H$_{arom}$), 82.31 (d, $^1J_{C\text{-}F}$ = 172.06 Hz, **C**H$_2$-F), 81.79 (**C**$_{tBu}$), 79.51 (**C**$_{tBu}$), 54.75 (**C**H$_\alpha$), 46.37 (d, $^2J_{C\text{-}F}$ = 21.60 Hz, **C**H$_2$CH$_2$-F), 28.34 (**C**H$_2$), 27.92 (**C**H$_3$). Mp: 168-171 °C (decomposition). MS: (ESI, positive): *m/z* = 407 [M+H], 429 [M+Na]. FT-MS (positive): *m/z* = 407.23406 (calculated), 407.23412 (measured) [M+H]. Anal. Calcd for $C_{22}H_{18}FN_5O_2$: C, 65.00; H, 7.69; N, 6.89. Found: C, 64.97; H, 7.71; N, 7.16.

5. Synthesis of 1-(2-fluoroethyl)-DL-tryptophan chlorhydrate (1-FETrp.HCl) (5.HCl).



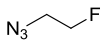
tert-Butoxycarbonyl-(1-(2-fluoroethyl)-tryptophan) *tert*-butyl ester (**4**) (160 mg, 0.39 mmol) in 1,4-dioxane (8 mL) and 6 N HCl (8 mL) was mixed 1h30 at room temperature. After solvents evaporation, the crude solid was washed with water and lyophilized to give the product **5**.HCl as a light brown powder (98.4 mg, 93%). ^{1}H NMR (250 MHz, D_2O) δ 7.64 (d, *J* = 7.8 Hz, 1H, CH_{arom}), 7.46 (d, *J* = 8.2 Hz, 1H, CH_{arom}), 7.28 (t, *J* = 7.3 Hz, 1H, CH_{arom}), 7.25 (s, 1H, CH_{indol}), 7.18 (t, *J* = 7.3 Hz, 1H, CH_{arom}), 4.73 (dt, $^2J_{H\text{-}F}$ = 48.26 Hz and $^3J_{H\text{-}H}$ = 4.24 Hz, 2H, C**H$_2$**-F), 4.42 (dt, $^3J_{H\text{-}F}$ = 28.95 Hz and $^3J_{H\text{-}H}$ = 4.44 Hz, 2H, C**H$_2$**-CH$_2$-F), 4.30 (dd, *J* = 7.0, 5.6 Hz, 1H, CH_α), 3.47 – 3.29 (m, 2H, CH_2). ^{13}C NMR (63 MHz, D_2O) δ 171.92 (**C**O), 136.39 (**C**$_{arom}$), 128.63 (**C**H$_{indol}$), 127.14 (**C**$_{arom}$), 122.23 (**C**H$_{arom}$), 119.70 (**C**H$_{arom}$), 118.54 (**C**H$_{arom}$), 110.12 (**C**H$_{arom}$), 106.29 (**C**$_{arom}$), 83.52 (d, $^1J_{C\text{-}F}$ = 165.19 Hz, **C**H$_2$-F), 53.35 (**C**H$_\alpha$), 45.98 (d, $^2J_{C\text{-}F}$ = 19.70 Hz, **C**H$_2$CH$_2$-F), 25.56 (**C**H$_2$). ^{19}F NMR (376 MHz, D_2O) δ -220.26 (tt, $^2J_{H\text{-}F}$ = 47.0 Hz, $^3J_{H\text{-}F}$ = 28.74 Hz, 1F). MS: (ESI, positive): *m/z* = 251 [M+H], 501 [2M+H]; (ESI, negative) *m/z* = 249 [M-H], 285 (100) and 287 (33) [M+Cl], 499 [2M-H]. FT-MS (positive): *m/z* = 251.11903 (calculated), 251.11900 (measured) [M+H]. Mp: 168-171 °C (decomposition). Anal. Calcd for $C_{13}H_{15}FN_2O_2$.HCl.2H_2O: C, 48.37; H, 6.26; N, 8.67. Found: C, 49.33; H, 5.56; N, 8.02.

6. Synthesis of *tert*-butoxycarbonyl-(1-propargyl)-tryptophan *tert*-butyl ester (7).



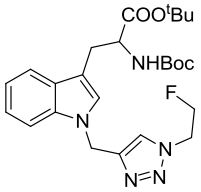
A solution of *tert*-butoxycarbonyl-L-tryptophan *tert*-butyl ester **2** (1.8012 g, 5 mmol) in DMF (20 mL) was stirred with sodium hydride (600 mg, 25 mmol) for 15 minutes at 0 °C and under N_2. Then, a solution of propargyl bromide (3 mL, 25 mmol, 80 %wt in toluene) was added dropwise and very slowly. After 5h, the solution was concentrated. The residue was finally purified by flash chromatography on silica gel (CH_3CN + 0.5% Et_3N) to give the product **7** as a yellow-brown oil (1,84 g, 4.62 mmol, 93%). R_f (Hexanes/EtOAc 4/1 (v/v)): 0.38; R_f (CH_2Cl_2/EtOAc 9/1 (v/v)): 0.87. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.58 (d, *J* = 7.8 Hz, 1H, CH_{arom}), 7.32 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 7.21 (t, *J* = 7.3 Hz, 1H, CH_{arom}), 7.10 (t, *J* = 7.3 Hz, 1H, CH_{arom}), 7.02 (s, 1H, CH_{indol}), 5.06 (d, *J* = 7.8 Hz, 1H, NH), 4.79 (d, *J* = 2.4 Hz, 2H, $CH_{2\ propar}$), 4.55 – 4.46 (m, 1H, CH_α), 3.29 – 3.12 (m, 2H, CH_2), 2.36 (d, *J* = 2.5 Hz, 1H, CH_{propar}), 1.40 (s, 9H, CH_3), 1.35 (s, 9H, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.32 (**C**O), 155.25 (**C**O), 135.96 (**C**$_{arom}$), 128.90 (**C**$_{arom}$), 125.83 (**C**H$_{indol}$), 122.06 (**C**H$_{arom}$), 119.60 (**C**H$_{arom}$), 119.48 (**C**H$_{arom}$), 110.31 (**C**$_{arom}$), 109.26 (**C**H$_{arom}$), 81.83 (**C**$_{tBu}$), 79.52 (**C**$_{tBu}$), 77.74 (**C**$_{propar}$), 73.60 (**C**H$_{propar}$), 54.73 (**C**H$_\alpha$), 35.66 (**C**H$_2$ propar), 28.36 (**C**H$_2$), 27.97 (**C**H$_3$). MS: (ESI, positive): *m/z* = 399 [M+H], 421 [M+Na], 797 [2M+H].

7. Synthesis of 1-azido-2-fluoroethane (8).



1-fluoro-2-tosyloxyethane (**3**) (4.37 g, 20 mmol) and sodium azide (1.63 g, 25 mmol) were solubilized in DMF (40 mL). The reaction was conducted 3h at 90 °C and additional 48h at 80 °C up to the complete consumption of **3** (monitoring by TLC (Hexane/EtOAc 9/1 (v/v))). Acetonitrile (60 mL) was then added and the precipitate was filtrated off. The resulting colorless solution was then used directly without further purification ([**8**] ≤ 0.2 mol/L). The yield of the crude product **8** was over 95%.

8. Synthesis of *tert*-butoxycarbonyl-(1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl))-tryptophan *tert*-butyl ester (9). Classical method with copper(I) salt.

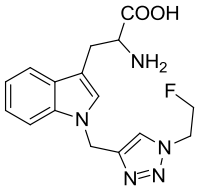


tert-Butoxycarbonyl-(1-propargyl)-tryptophan *tert*-butyl ester (**7**) (371 mg, 0.93 mmol) in DMF (5 mL) was shaken 5 minutes under N_2. Then, a solution containing tetrakis(acetonitrile)copper(I) hexafluorophosphate (40 mg, 10 mol%), **8** (7 mL, [**8**] ≤ 0,2 mol/L) and diisopropylethylamine (400 µL) in DMF (4 mL) was added dropwise. After 7h under N_2, the reaction was quenched with NH_4Cl (5 %wt in water). This aqueous solution was extracted 3 times with EtOAc. The organic layers were combined, dried over anhydrous $MgSO_4$ and evaporated to dryness. The residue was finally purified by chromatography on silica gel (CH_2Cl_2/EtOAc 9/1 + 0.5% Et_3N (v/v)) to give **9** as a yellow oil (367 mg, 0.76 mmol, 81%). R_f (Hexanes/EtOAc 4/1 (v/v)): 0.04; R_f (CH2Cl2/EtOAC 9/1 (v/v)): 0.42. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.33 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.28 (s, 1H, $CH_{triazole}$), 7.18 (t, *J* = 7.0 Hz, 1H, CH_{arom}), 7.10 (t, *J* = 7.2 Hz, 1H, CH_{arom}), 7.00 (s, 1H, CH_{indol}), 5.38 (s, 2H, $CH_{2\ propar}$), 5.08 (d, *J* = 7.8 Hz, 1H, NH), 4.71 (dt, $^2J_{H\text{-}F}$ = *46.7 Hz* and $^3J_{H\text{-}H}$ = 4.5 Hz, 2H, C**H$_2$**-F), 4.53 (dt, $^3J_{H\text{-}F}$ = *26.6 Hz* and $^3J_{H\text{-}H}$ = 5.4 Hz, 2H, C**H$_2$**CH$_2$-F), 4.55 – 4.49 (m, 1H, CH_α), 3.21 (qd, *J* = 14.6, 5.2 Hz, 2H, CH_2), 1.39 (s, 9H, CH_3), 1.35 (s, 9H, CH_3). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.33 (CO), 155.25 (CO), 145.27 ($C_{triazole}$) 136.11 (C_{arom}), 128.88 (C_{arom}), 126.40 (CH_{indol}), 122.75 ($CH_{triazole}$), 122.12 (CH_{arom}), 119.53 (CH_{arom}), 119.39 (CH_{arom}), 110.54 (C_{arom}), 109.45 (CH_{arom}), 81.31 (d, $^1J_{C\text{-}F}$ = 172.95 Hz, CH_2-F), 81.91 (C_{tBu}), 79.58 (C_{tBu}), 55.01 (CH_α), 50.58 (d, $^2J_{C\text{-}F}$ = 20.71 Hz, CH_2CH_2-F), 41.95 ($CH_{2\ propar}$), 28.37 (CH_2), 28.00 (CH_3). MS: (ESI, positive): *m/z* = 488 [M+H]. FT-MS (positive): *m/z* = 488.266759 (calculated), 488.266662 (measured) [M+H].

9. Synthesis of *tert*-butoxycarbonyl-(1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl))-tryptophan *tert*-butyl ester (9). Method with copper(I) supported resin.

tert-Butoxycarbonyl-(1-propargyl)-tryptophan *tert*-butyl ester (**7**) (80 mg, 0.2 mmol) was solubilized in a solution of 8 in acetonitrile (5 mL; [**8**] ≤ 0,2 mol/L). The solution was slowly shaken in presence of Amberlyst A-21.CuI resin (15 mg) for 48h at room temperature. Then, 50 mg of Quadrapure^{TM} TU were added to eliminate copper potentially liberated during the reaction, and the reaction was shaken two additional hours. Resins were filtrated and washed with CH_2Cl_2. The solution was concentrated and purified by chromatography on silica gel (CH_2Cl_2/EtOAc 9/1 (v/v) + 0,5% Et_3N) to give pure **9** (70 mg, 0.146 mmol, 73%) as a yellow oil.

10. Synthesis of 1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-tryptophan chlorhydrate (10.HCl).



To *tert*-butoxycarbonyl-(1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl))-tryptophan *tert*-butyl ester (**9**) (20 mg, 0.041 mmol) in 1,4-dioxane (3 mL) was added 6 N HCl (3 mL). After 4h of stirring at room temperature, the solvents were evaporated and the crude solid was washed with water and lyophilized to give **10**.HCl as an off white powder (12.5 mg, 83%). R_f (CH_2Cl_2/EtOAC 9/1 (v/v)): 0.0. ^{1}H NMR (250 MHz, D_2O) δ 7.99 (s, 1H, $CH_{triazole}$), 7.71 (d, *J* = 7.9 Hz, 1H, CH_{arom}), 7.50 (d, *J* = 8.1 Hz, 1H, CH_{arom}), 7.38 (s, 1H, CH_{indol}), 7.31 (ddd, *J* = 8.3, 7.2, 1.2 Hz, 1H, CH_{arom}), 7.21 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H, CH_{arom}), 5.48 (s, 2H, $CH_{2\ propar}$), 4.91 – 4.67 (d, $^2J_{H\text{-}F}$ = 46.9 Hz, 2H, C**H$_2$**-F), 4.77 – 4.60 (d, $^3J_{H\text{-}F}$ = 28.2 Hz, 2H, C**H$_2$**CH$_2$-F), 4.30 (dd, *J* = 7.3, 5.4 Hz, 1H, CH_α), 3.45 (qd, *J* = 15.2, 6.6 Hz, 1H, CH_2). ^{13}C NMR (63 MHz, D_2O) δ 172.40 (**C**OOH), 144.38 (**C**$_{triazole}$), 136.02 (**C**$_{arom}$), 128.46 (**C**H$_{indol}$), 127.47 (**C**$_{arom}$), 124.34 (**C**H$_{triazole}$), 122.41 (**C**H$_{arom}$), 119.90 (**C**H$_{arom}$), 118.73 (**C**H$_{arom}$), 110.19 (**C**H$_{arom}$), 106.94 (**C**$_{arom}$), 81.91 (d, $^1J_{C\text{-}F}$ = 167.5 Hz, **C**H$_2$-F), 53.65 (**C**H$_\alpha$), 50.66 (d, $^2J_{C\text{-}F}$ = 19.6 Hz, **C**H$_2$CH$_2$-F), 40.70 (**C**H$_{2\ propar}$), 25.74 (**C**-H$_2$). ^{19}F NMR (376 MHz, D_2O) δ -222.50 (tt, $^2J_{H\text{-}F}$ = 46.5 Hz, $^3J_{H\text{-}F}$ = 28.2 Hz, 1F). MS: (ESI, positive): *m/z* = 332 [M+H], 369 [M+HCl+H], 387; ESI, negative): *m/z* = 330 [M-H]. FT-MS (positive): *m/z* = 332.151730 (calculated), 332.151738 (measured) [M+H]. Anal. Calcd for $C_{16}H_{18}FN_5O_2$.HCl.2H_2O: C, 47.58; H, 5.75; N, 17.34. Found: C, 48.36; H, 5.24; N, 17.46.

2. Enzymatic Assays

1. rhIDO: Methylene blue/L-ascorbic acid assays – Generality

In vitro enzymatic assays were carried out in presence of rhIDO (synthesized and purified by the Center for Protein Engineering; concentration depending of the test(s) realized), methylene blue (10 µM), L-ascorbic acid (20 mM), catalase from bovine liver (Sigma, 26100 U.mg^{-1}, 0.1 mg.mL^{-1}) and BSA (bovine serum albumin; 0.1 mg.mL^{-1}) in sodium phosphate buffer (50 mM, pH 6.5) at 37 °C for several incubation times. As mentioned by Sono in 1989, the presence of methylene blue and L-ascorbic acid is essential for these *in vitro* oxidations catalyzed by rhIDO.[3] Indeed, L-ascorbic acid is a good antioxidant, maintaining the iron atom in its ferrous state (Fe^{2+}), as found in the active form of the enzyme. In addition, methylene blue allows the electron transfer from L-ascorbic acid to the heme of the enzyme. Catalase is also added to avoid the rhIDO inactivation caused by hydrogen peroxide produced in the enzymatic test as a by-product.[3-4] The reaction was initiated by addition of L-tryptophan (or tryptophan analogues) (total volume: 100 µL).

2. Measurement of L-kynurenine (and analogues) by HPLC (or UPLC) analysis using UV detection (method A) or fluorescence detection (method B).

After different times of incubation, the reaction was stopped by addition of perchloric acid (30 %wt, 20 µL) and again incubated for fifteen (or thirty minutes for the N^1-alkylated substrates) at 65 °C, to form L-kynurenine or kynurenine derivatives. After centrifugation for 10 minutes at 14600 rpm, the solution was analyzed on a HPLC RP18 X-Terra (4.6 x 150 mm, 3.5 µm) column, or on an ACQUITY UPLC® BEH C18 1.7 µm column, 2.1 x 100 mm (Waters). The analyses were performed in CH_3CN/ammonium acetate (10 mM) 10/90 (v/v) (flow: 0.7 mL/min). Under these conditions, L-tryptophan and analogues were detected either with an UV PDA detector (method A) or with a fluorescence detector (2475 fluorescence (FLR) detector for modular LC systems; excitation: 270 nm and emission: 350 nm) (method B). Method A allowed the determination of L-tryptophan (and tryptophan analogues) at 221 and 284 nm, while kynurenine derivatives were measured at 321 nm and 360 nm. On the other hand, only L-tryptophan (or tryptophan derivatives) concentration was determined with method B. The HPLC retention times of L-tryptophan, *N*-formyl-L-kynurenine and L-kynurenine (and their analogues) obtained under these elution conditions were summarized in Table 3 (see "*5. UPLC-MS Analysis*" for UPLC spectra).

3. Comparison of several potential substrates.

The concentration of rhIDO was varied from 0.1 µM to 5 µM. These *in vitro* enzymatic reactions were tested for two concentrations of substrate (100 µM and 1 mM) and were stopped after different incubation times (1h or 4h) and analyzed either by method A ([S] = 1 mM), or by method B ([S] = 100 µM) as described above.

4. K_m value determination for 1-FETrp (5).

The enzymatic assay was realized with a rhIDO concentration of 0.5 µM and in presence of various concentrations of 1-FETrp (from 0.5 to 200 µM). The reaction was stopped after 5, 10 or 15 minutes and analyzed by HPLC (method B) as previously described.

5. Determination of k_{cat}/K_m ratio values.

The k_{cat}/K_m ratio is a second order constant characterizing the reaction rate when the substrate concentration is smaller than K_m. Indeed, for these concentrations, the initial rate (v_0) is described in Equation 1.

Equation 1

$$v_0 = \frac{k_{cat} E_0}{K_m}[S]$$

With [S] >> E_0, and [S] < K_m.

For a first order reaction, the decrease of the substrate concentration in function of the time can be described by Equation 2 and was determinate via the fluorescence detection method.

Equation 2

$$[S] = [S]_0 e^{\frac{-k_{cat} E_0 t}{K_m}}$$

These kinetic studies were performed with **12** (30 µM), **13** (10 µM), **5** (40 µM). These substrates were incubated as described before with the following rhIDO concentrations (**12**: 0.25 µM, **13**: 0.5 µM, **5**: 2.5 µM), in sodium phosphate buffer at 37 °C. The reactions were stopped after 0, 4, 8, 12, 16, 20 and 24 minutes and the solutions were analyzed by method B as described previously.

a. Calibration curve for 5: HPLC chromatograms superposition:

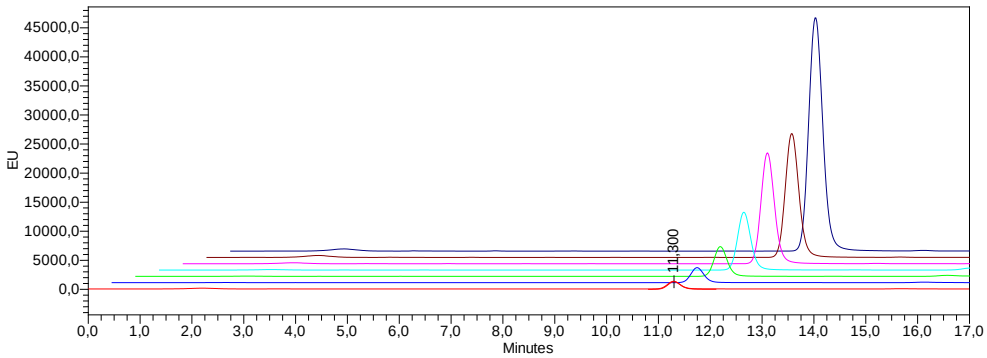


Figure S 1. HPLC chromatograms superposition obtained by fluorescence detection – Calibration curve.
5 concentrations : 5 µM (red), 10 µM (blue), 20 µM (green), 40 µM (light blue), 80 µM (pink), 100 µM (brown) and 200 µM (dark blue).

b. Enzymatic assays with rhIDO: HPLC chromatograms superposition:

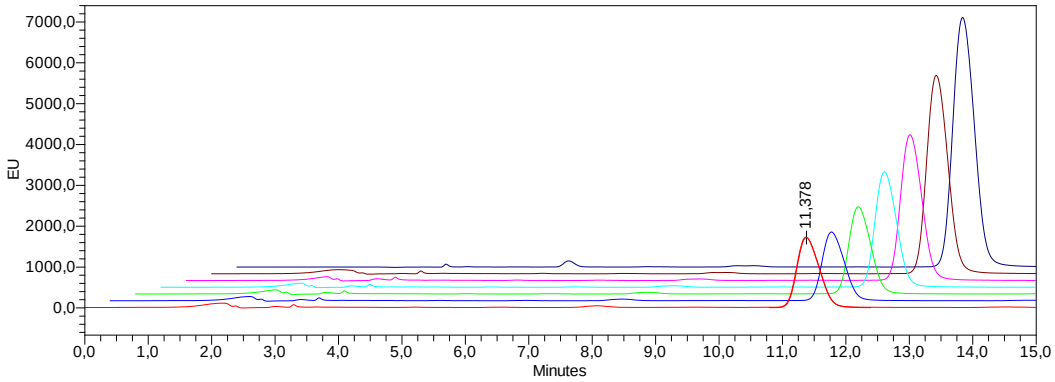


Figure S 2. HPLC chromatograms superposition obtained by fluorescence detection and after different incubation times. Assays realized in presence of **5** (40 µM) and rhIDO (2.5 µM). The reaction is quenched after different incubation times: 24 min (red), 20 min µM (blue), 16 min (green), 12 min (light blue), 8 min (pink), 4 min (brown) and 0 min (dark blue).

c. k_{cat}/K_m determination for 1-FETrp (5)

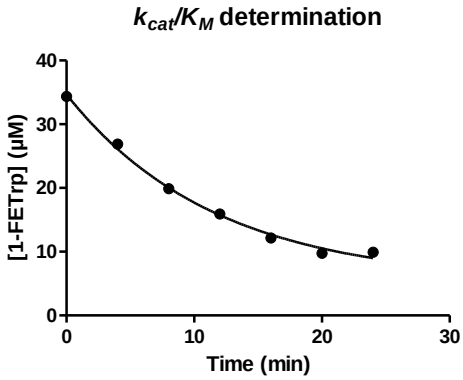


Figure S 3. Diminution of the substrate concentration in function of the incubation time.

6. In vitro enzymatic assays with rhTDO.

In vitro enzymatic assays were carried out in presence of rhTDO recombinant human tryptophan 2,3-dioxygenase (rhTDO; synthesized and purified by the Center for Protein Engineering; 0.1 or 1 µM depending of the test(s) realized), methylene methylene blue (10 µM), L-ascorbic acid (20 mM), catalase (Sigma, 26100 U.mg^{-1}, 0.1 mg.mL^{-1}) and bovine hemin (10 µM) in Tris buffer (50 mM, pH 7.5).[5-6] The reaction was initiated by the addition of L-tryptophan (**11**) (or analogues (**12**, **13** and **5**)). After one hour of incubation at 37 °C, the enzymatic reaction was quenched by an addition of perchloric acid (30 %wt, 20 µL) and incubated again for fifteen minutes at 65 °C. After centrifugation for 10 minutes at 14600 rpm, the solution was then analyzed by HPLC with fluorescence detection (method B) as described previously.

3. REFERENCES

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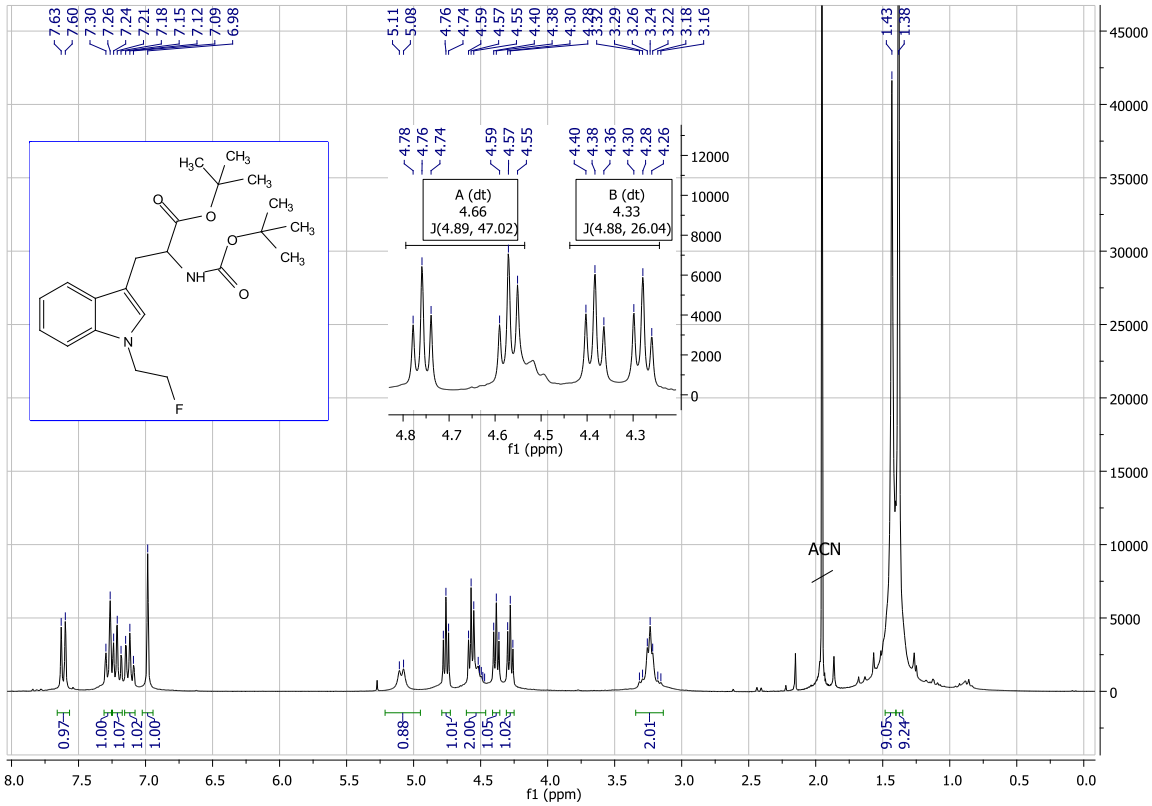
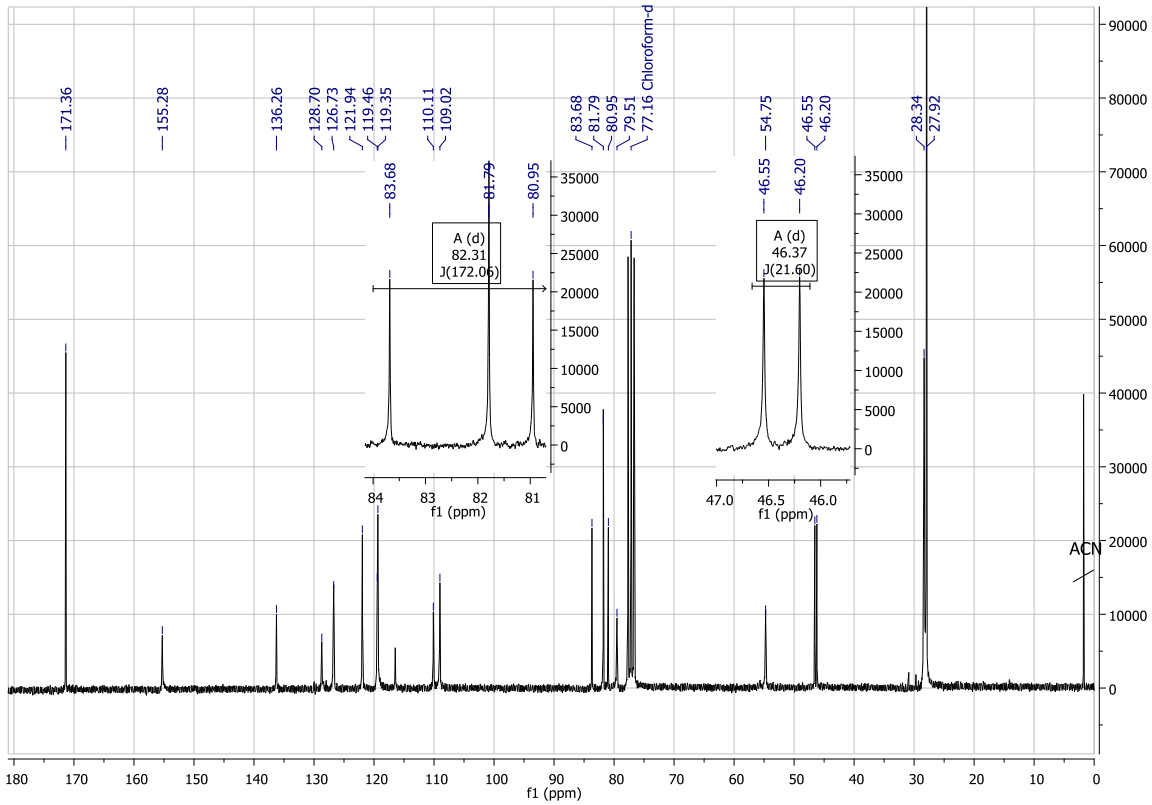
4. ^{1}H and ^{13}C NMR of compounds 4, 5, 9 and 10

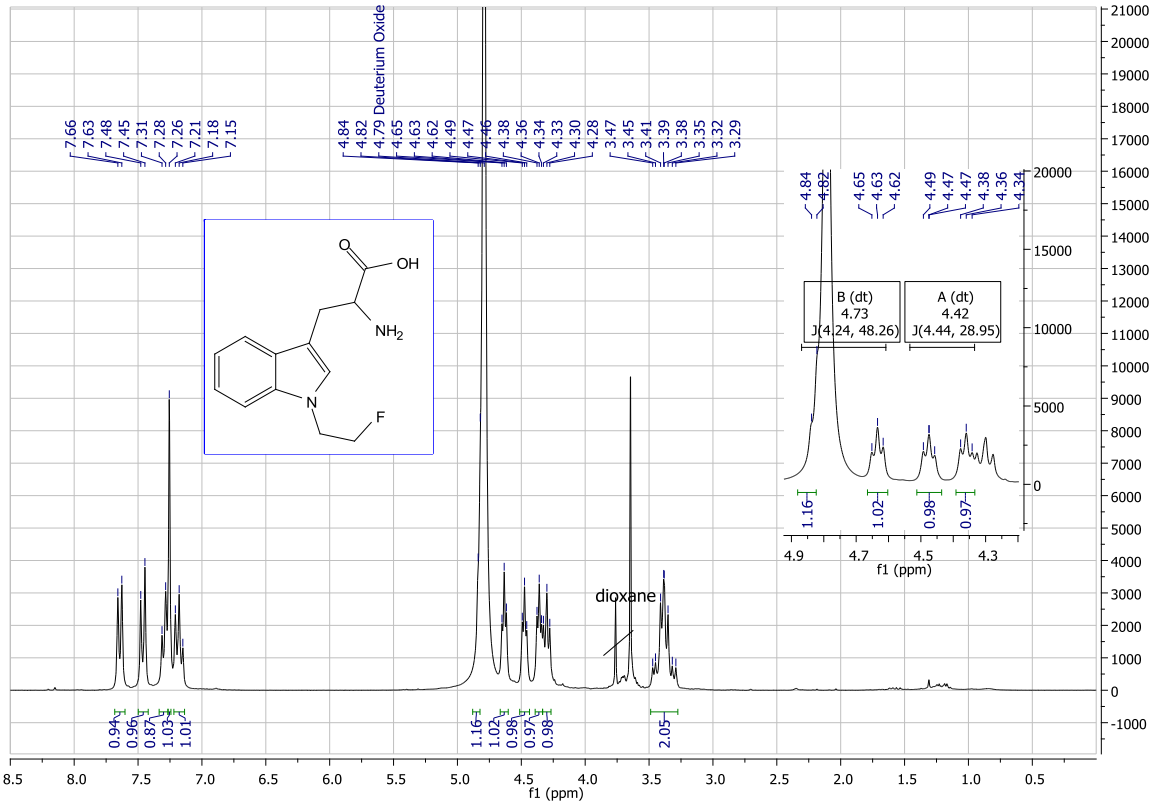
1. ^{1}H spectrum of *tert*-butoxycarbonyl-(1-(2-fluoroethyl)-tryptophan) *tert*-butyl ester (4) – 250 MHz, $CDCl_3$-d_1



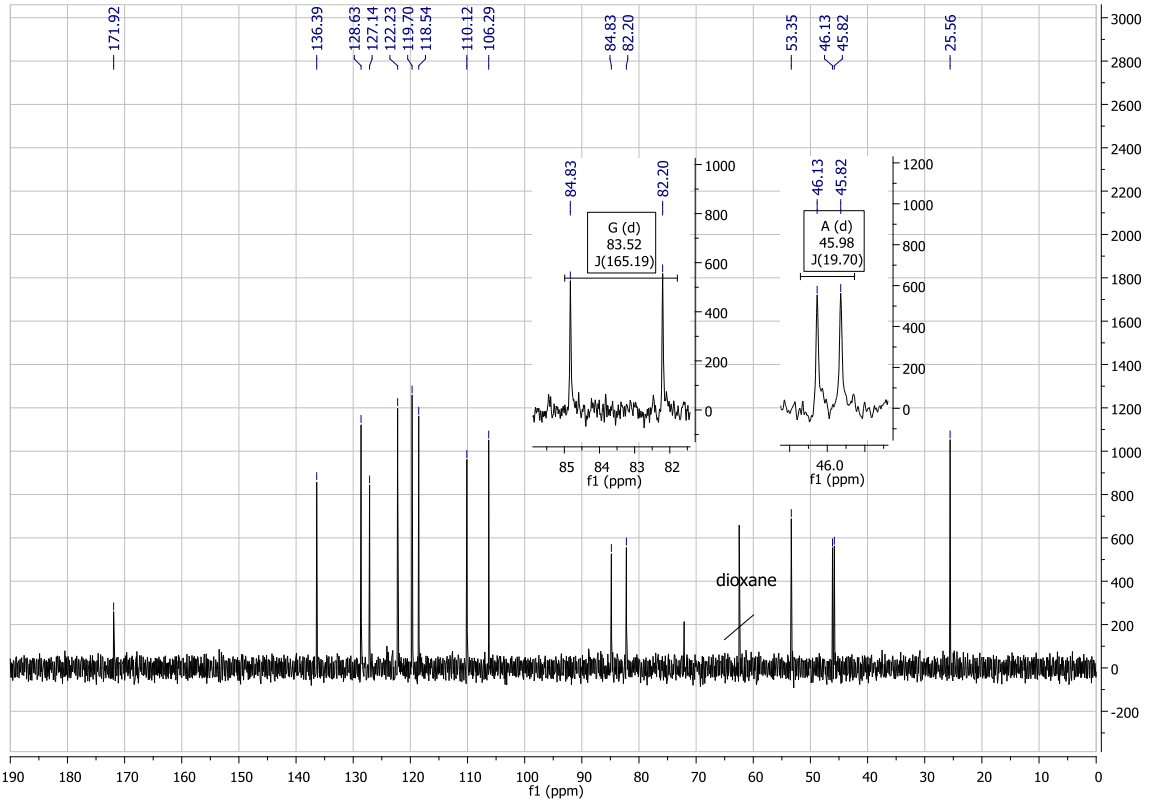
2. ^{13}C spectrum of *tert*-butoxycarbonyl-(1-(2-fluoroethyl)-tryptophan) *tert*-butyl ester (4) – 250 MHz, $CDCl_3$-d_1



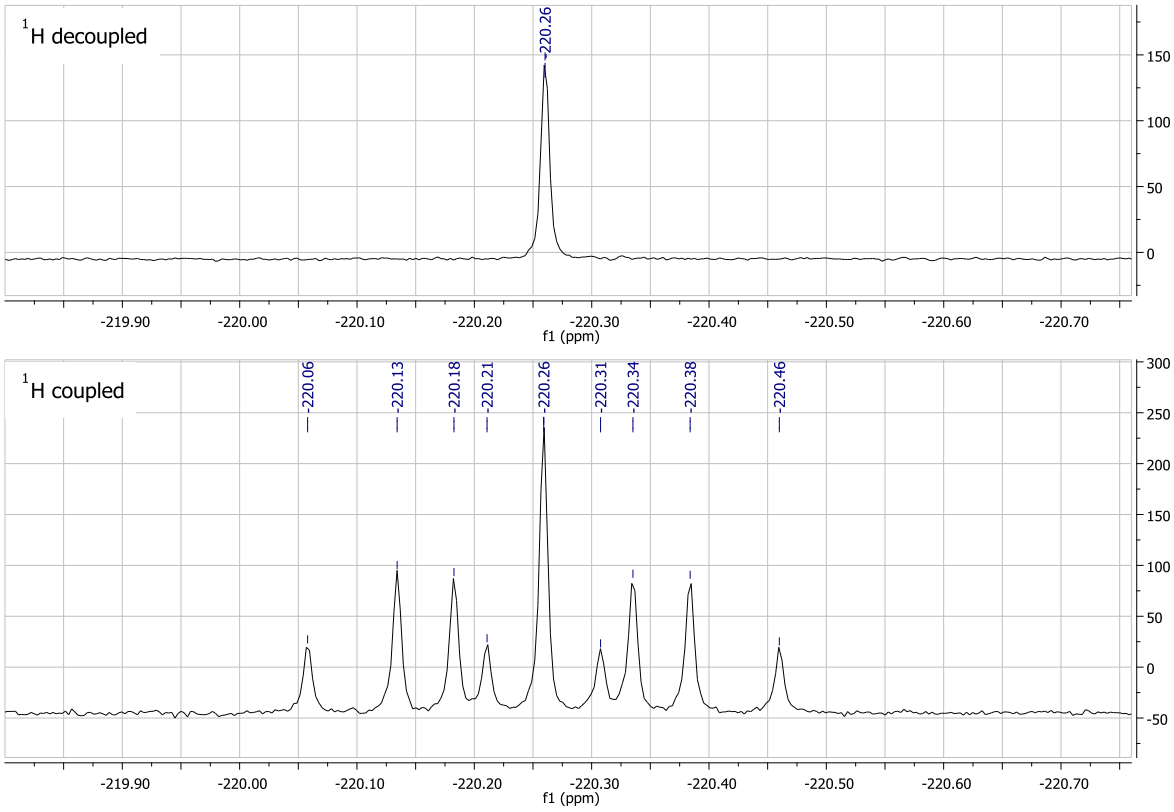
3. ^{1}H spectrum of 1-(2-fluoroethyl)-tryptophan (5) – 250 MHz, D_2O-d_2



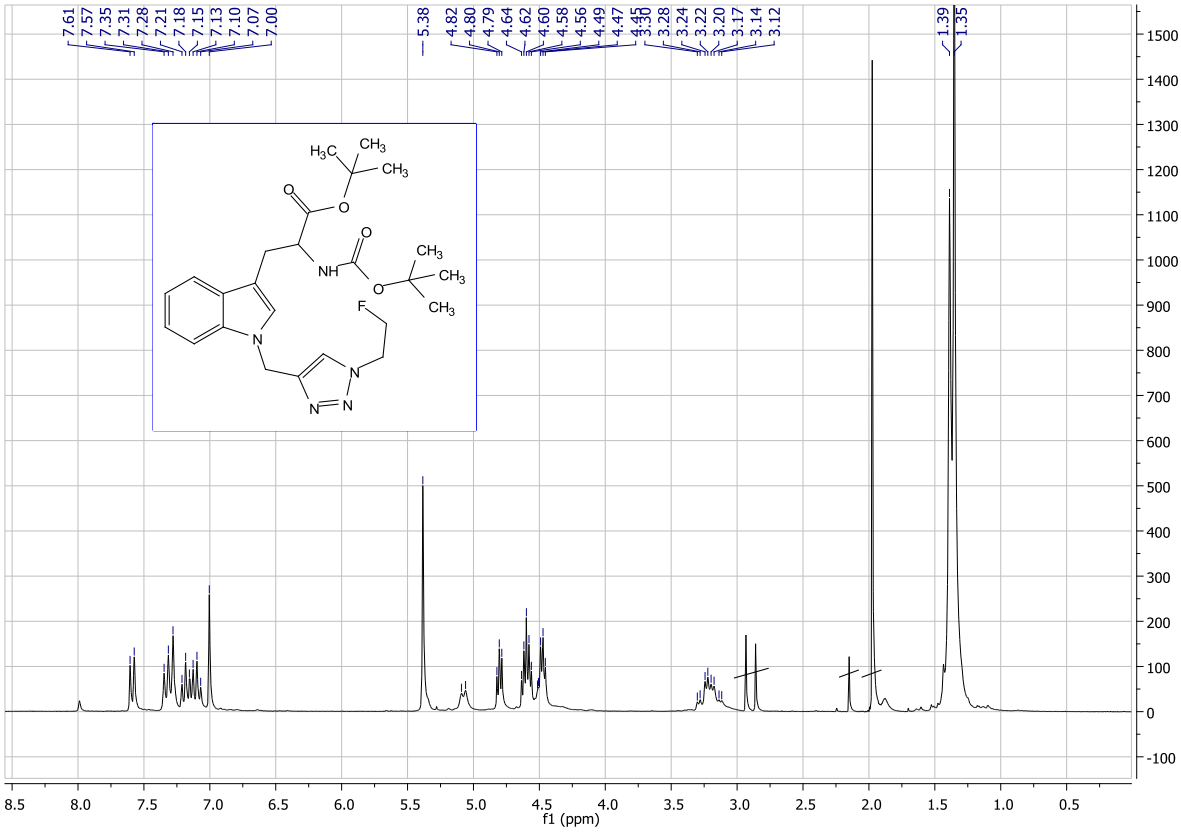
4. ^{13}C spectrum of 1-(2-fluoroethyl)-tryptophan (5) – 250 MHz, D_2O-d_2



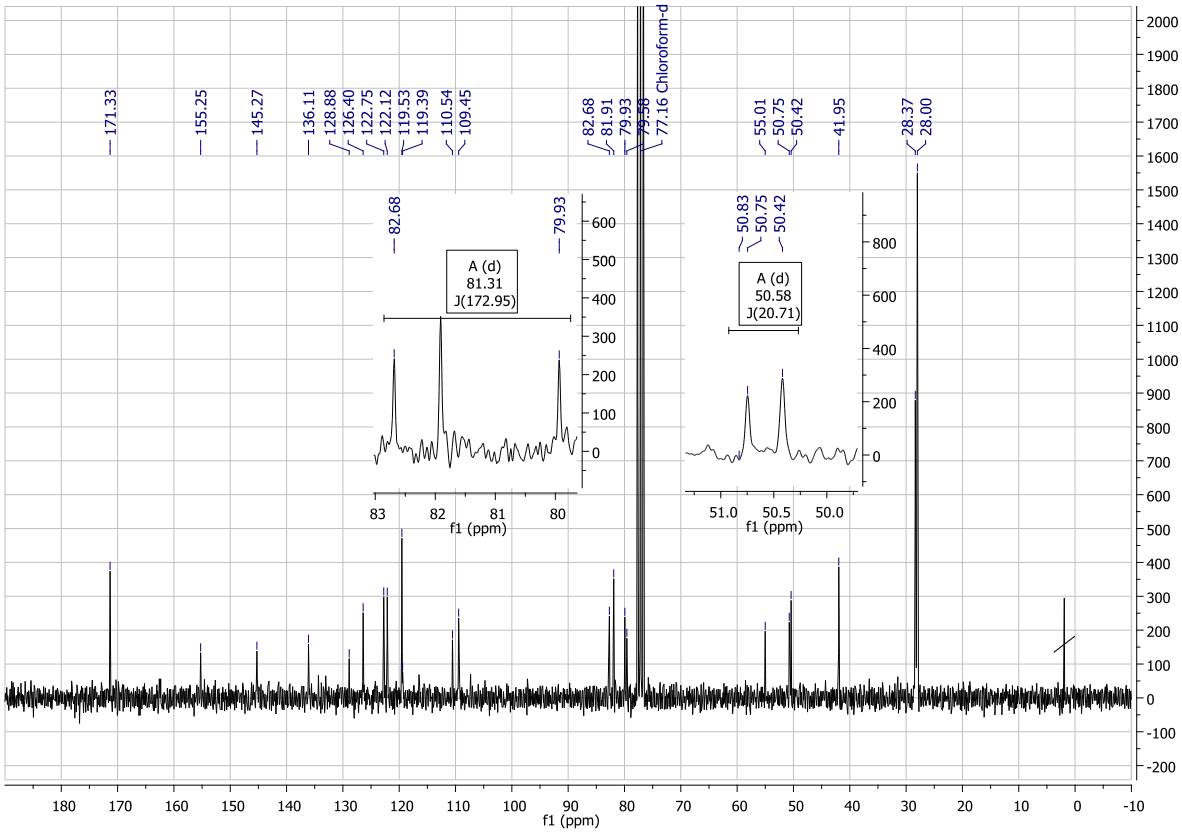
5. ^{19}F spectra of 1-(2-fluoroethyl)-tryptophan (5) – 400 MHz, D_2O-d_2



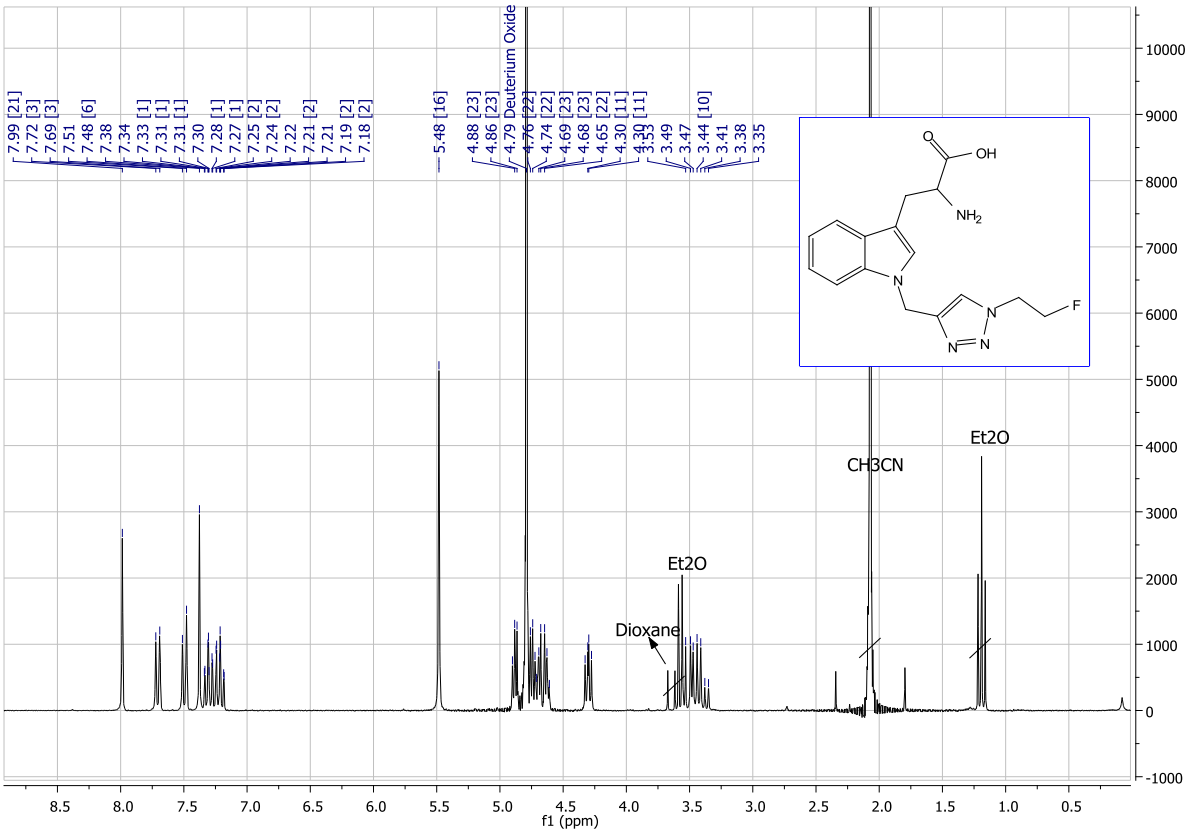
6. ^{1}H spectrum of *tert*-butoxycarbonyl-(1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl))-tryptophan *tert*-butyl ester (9) – 250 MHz, $CDCl_3$-d_1



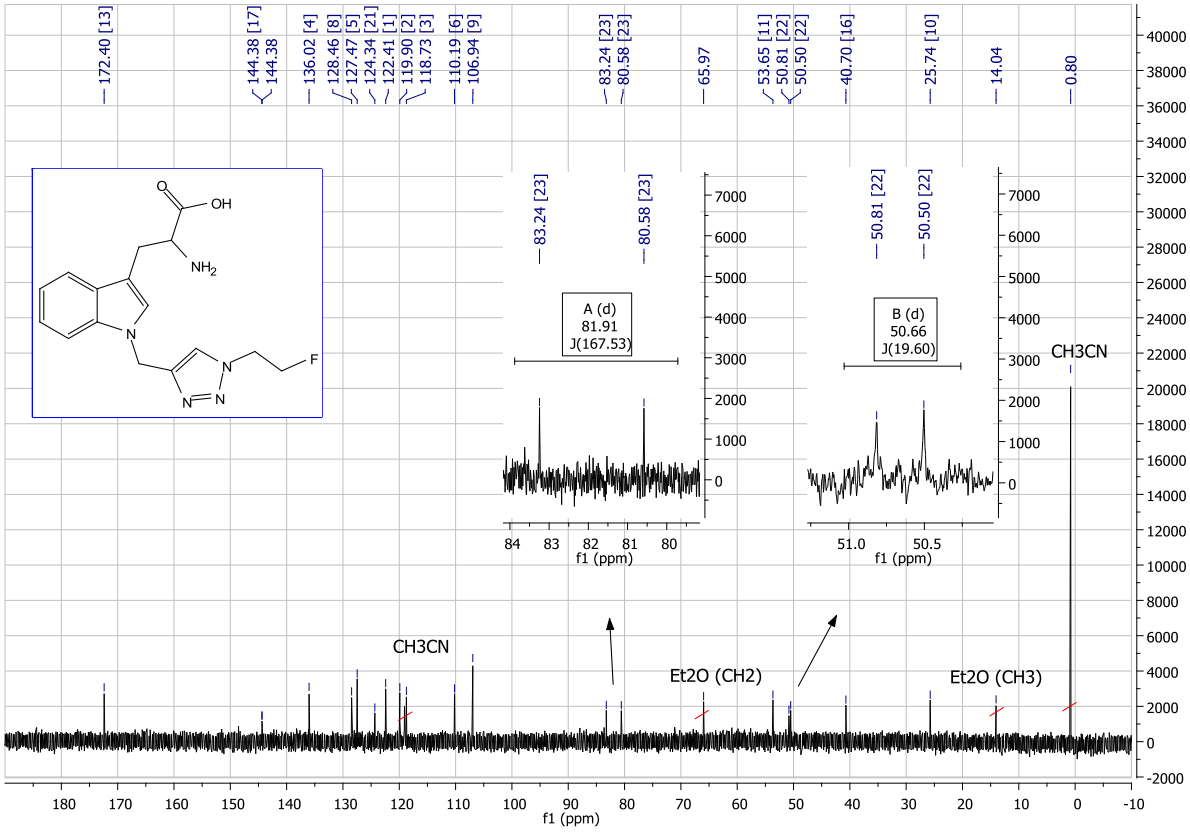
7. ^{13}C spectrum of *tert*-butoxycarbonyl-(1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl))-tryptophan *tert*-butyl ester (9) – 250 MHz, $CDCl_3$-d_1



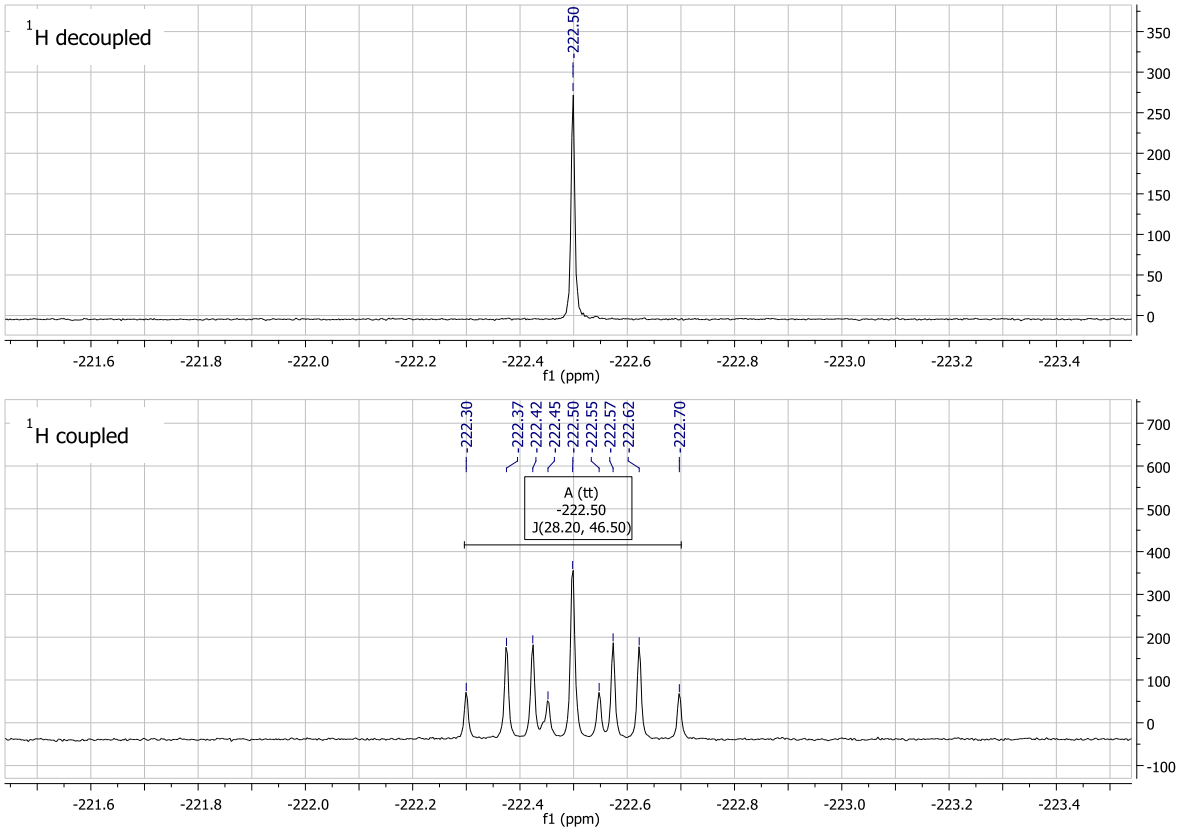
8. ^{1}H spectrum of 1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-tryptophan (10) – 250 MHz, D_2O-d_2



9. ^{13}C spectrum of 1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-tryptophan (10) – 250 MHz, D_2O-d_2



10. ^{19}F spectra of 1-((1-(2-fluoroethyl)-*1H*-1,2,3-triazol-4-yl)methyl)-tryptophan (10) – 400 MHz, D_2O-d_2



5. UPLC-MS Analysis

1. Nature of the kynurenine derivatives confirmation by Mass Spectrometry.

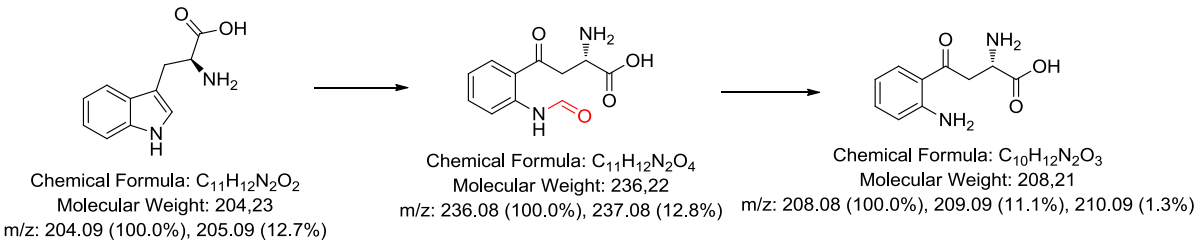
The different potential substrates were incubated 1h with rhIDO at 37 °C, hydrolyzed with perchloric acid (30 %wt, 20 µL) and incubated at 65 °C for fifteen minutes or thirty minutes for N^1-alkylated substrates. Then, HPLC and UPLC analyses (method A) were done and two product peaks appeared on the chromatograms for **11**, **12**, **13** and **5** (see below).

2. UPLC conditions:

- Column: ACQUITY UPLC® BEH C18 1.7 µm column, 2.1 x 100 mm (Waters)
- Pre-column: ACQUITY UPLC® BEH C18 1.7 µm VanGuard™ pre-column, 2.1 x 5 mm (Waters)
- Eluent: NH_4Ac buffer 10mM pH~6.5/CH_3CN (90/10) (except for **13** (98/2)) (v/v)
- Flow: 0.7 mL/min
- Temperature: 50 °C
- Injected volume: 2 µL
- Detection wavelength: 221 nm et 360 nm

3. Structures and UPLC spectra of substrates and products:

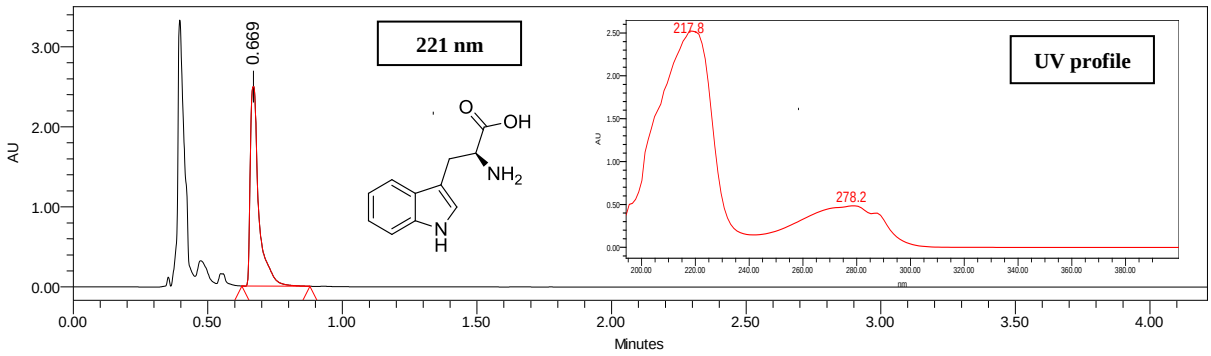
a. *L*-Trp (*L*-tryptophan) (11):



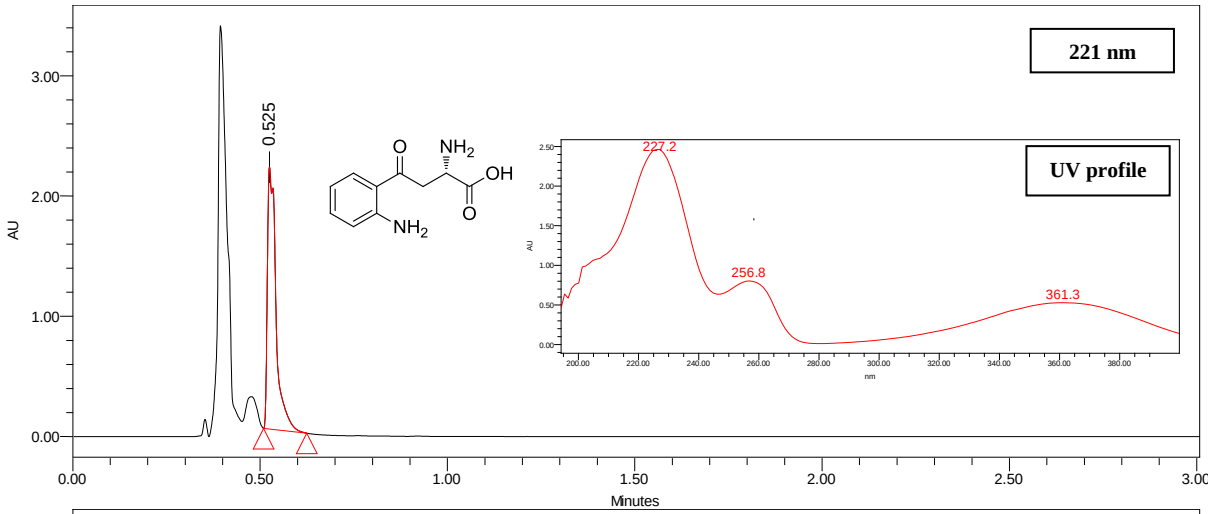
Retention time: 0.67 min – **0.52 min**

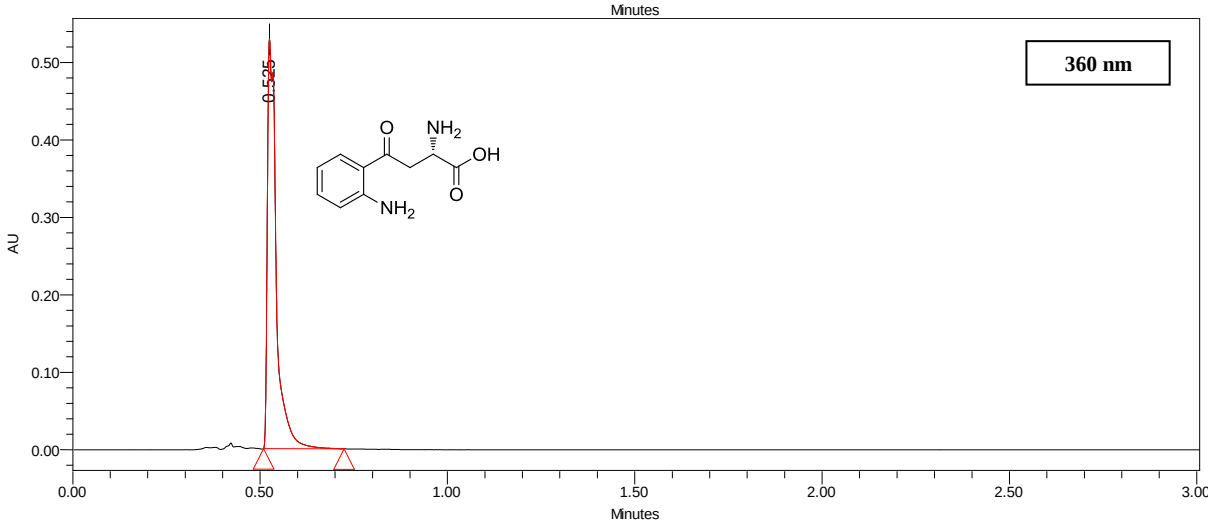
<u>Remarque:</u> The *N*-formyl-L-kynurenine derivative has a formyl groupment (in red), easily cleavable in acidic media. This compound is thus not observable in UPLC spectra.

UPLC spectrum of *L*-tryptophan reference:

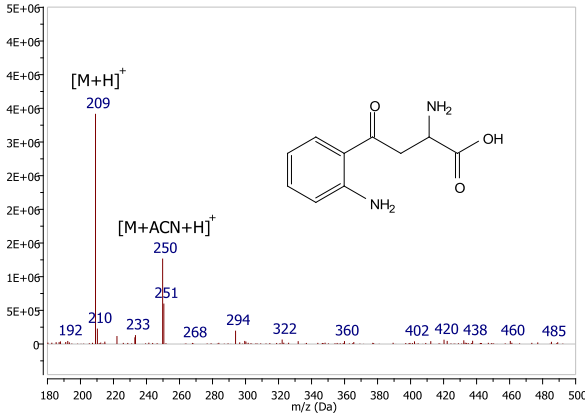


UPLC spectrum obtained after *in vitro* enzymatic assays with L-tryptophan and rhIDO and hydrolysis with perchloric acid:

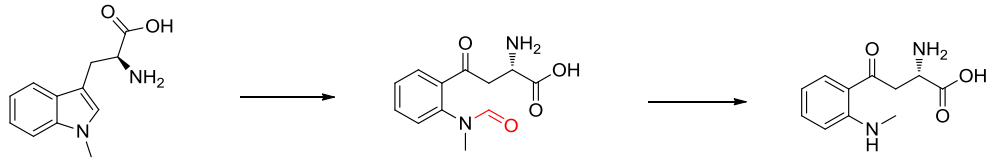




Mass Spectra of the collected peak obtained at 0.52 min



b. 1-Me-*L*-Trp (1-methyl-L-tryptophan) (12):



Chemical Formula: $C_{12}H_{14}N_2O_2$
Molecular Weight: 218,25
m/z: 218.11 (100.0%), 219.11 (13.2%), 220.11 (1.3%)

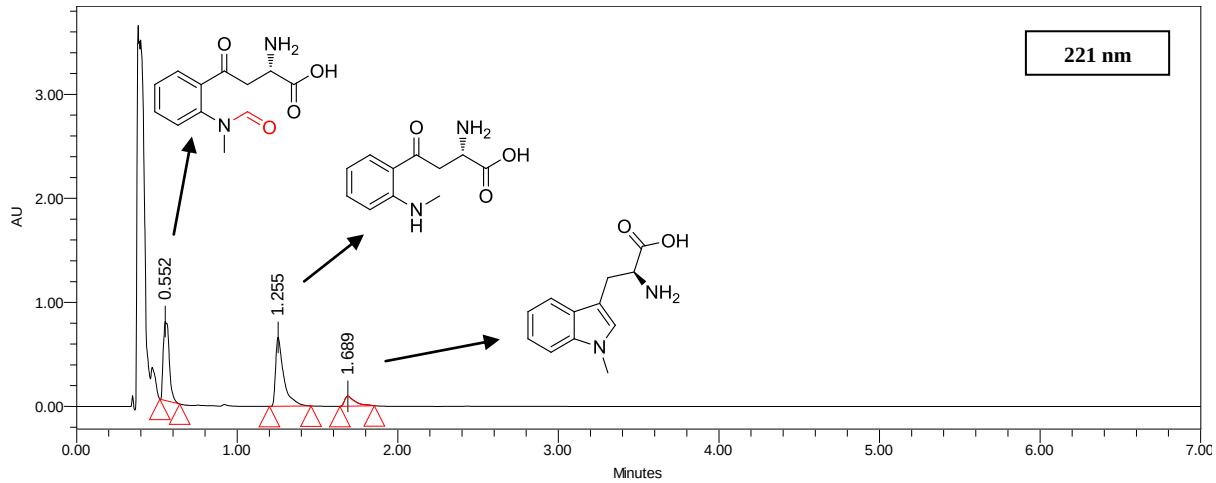
Chemical Formula: $C_{12}H_{14}N_2O_4$
Molecular Weight: 250,25
m/z: 250.10 (100.0%), 251.10 (13.3%), 252.10 (1.7%)

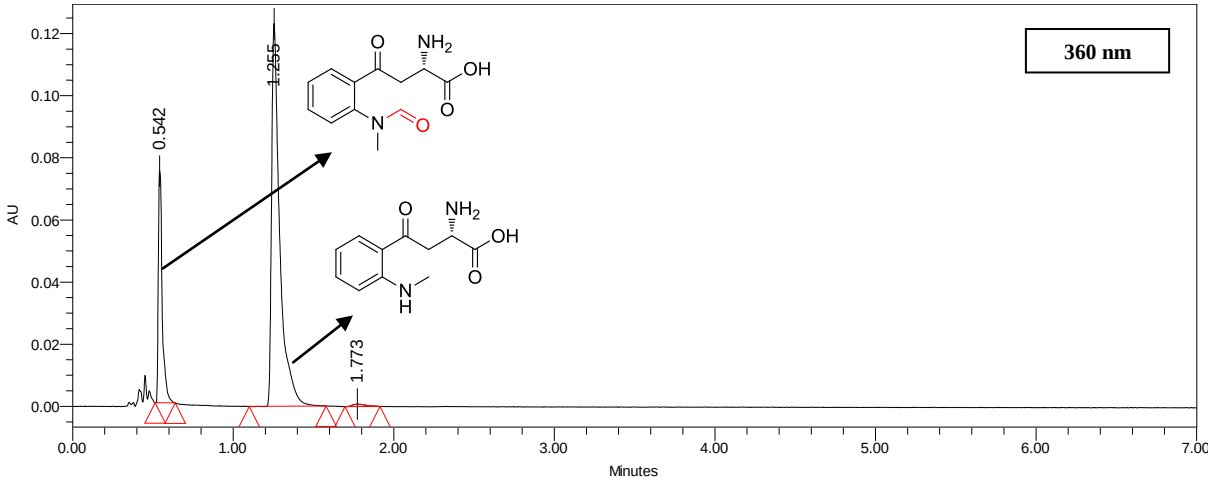
Chemical Formula: $C_{11}H_{14}N_2O_3$
Molecular Weight: 222,24
m/z: 222.10 (100.0%), 223.10 (12.8%)

Retention time: 1.69 min **0.55 min** **1.25 min**

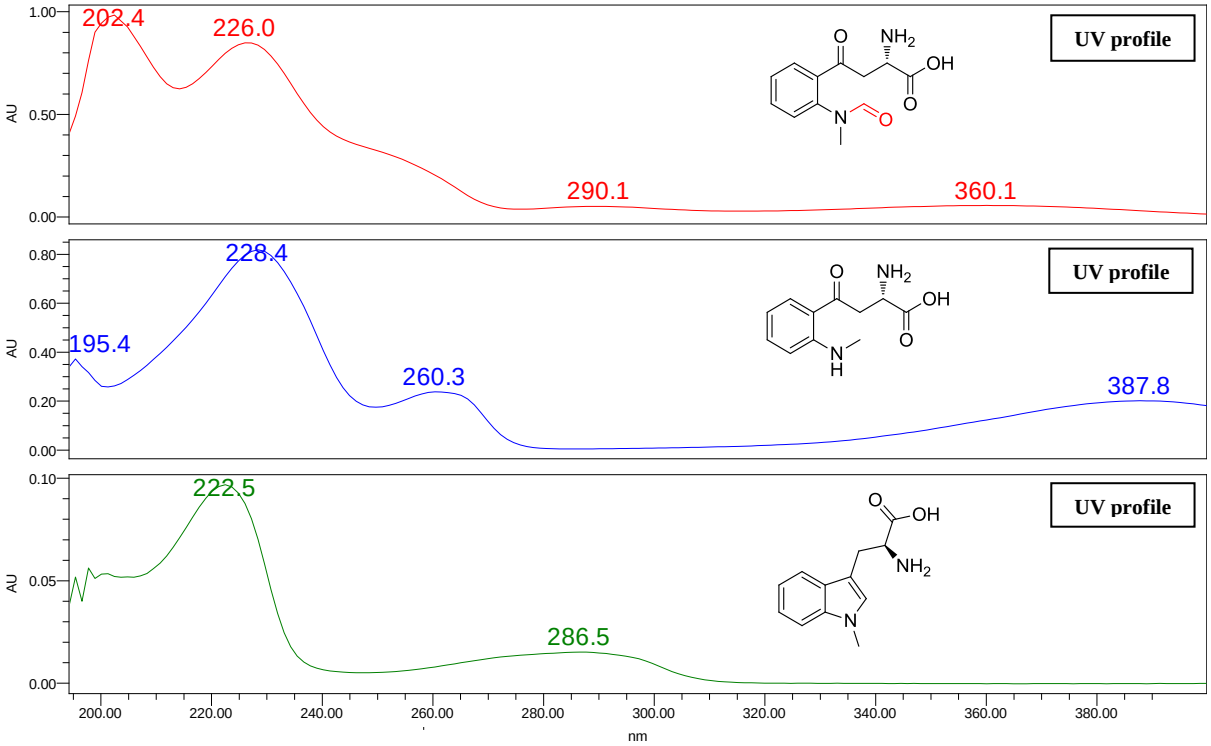
<u>Remarque:</u> The *N*-formyl-kynurenine derivative has a formyl groupment (in red), less easily cleavable in acidic media. This compound is thus observable in UPLC spectra.

UPLC spectrum obtained after *in vitro* enzymatic assays with 1-methyl-L-tryptophan and rhIDO followed by hydrolysis with perchloric acid:

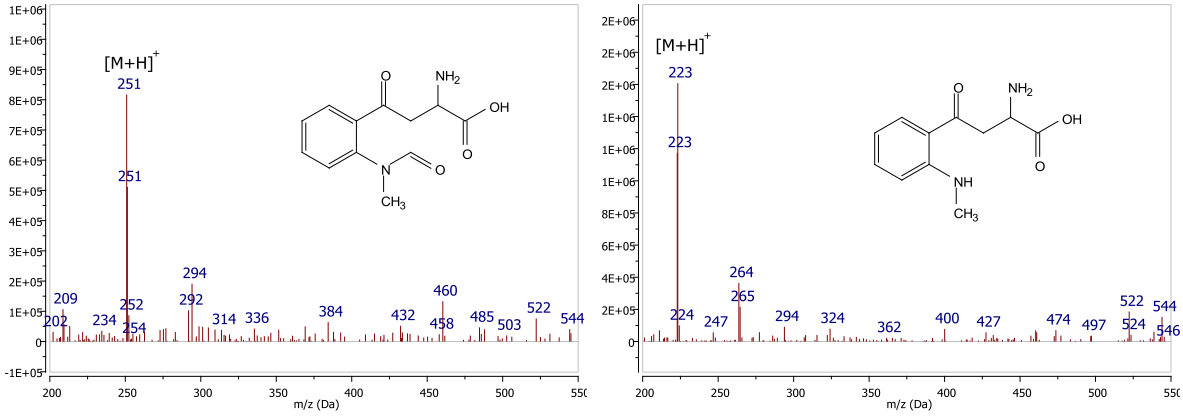




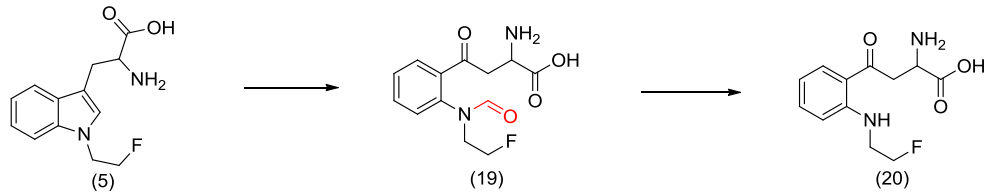
UV profile of integrated peaks



Mass Spectra of the collected peak obtained at 0.55 min and 1.25 min respectively



c. 1-FETrp (1-(2-fluoroethyl)-tryptophan) (5):



(5)
Chemical Formula: $C_{13}H_{15}FN_2O_2$
Molecular Weight: 250,27
m/z: 250.11 (100.0%),
251.12 (14.3%), 252.12 (1.4%)

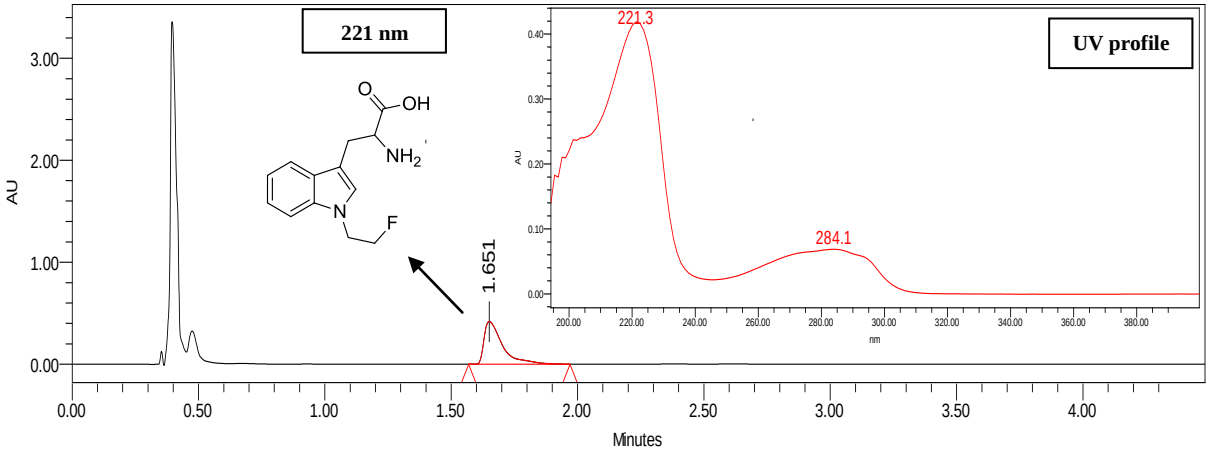
(19)
Chemical Formula: $C_{13}H_{15}FN_2O_4$
Molecular Weight: 282,27
m/z: 282.10 (100.0%),
283.10 (14.8%), 284.11 (1.8%)

(20)
Chemical Formula: $C_{12}H_{15}FN_2O_3$
Molecular Weight: 254,26
m/z: 254.11 (100.0%),
255.11 (13.3%), 256.11 (1.5%)

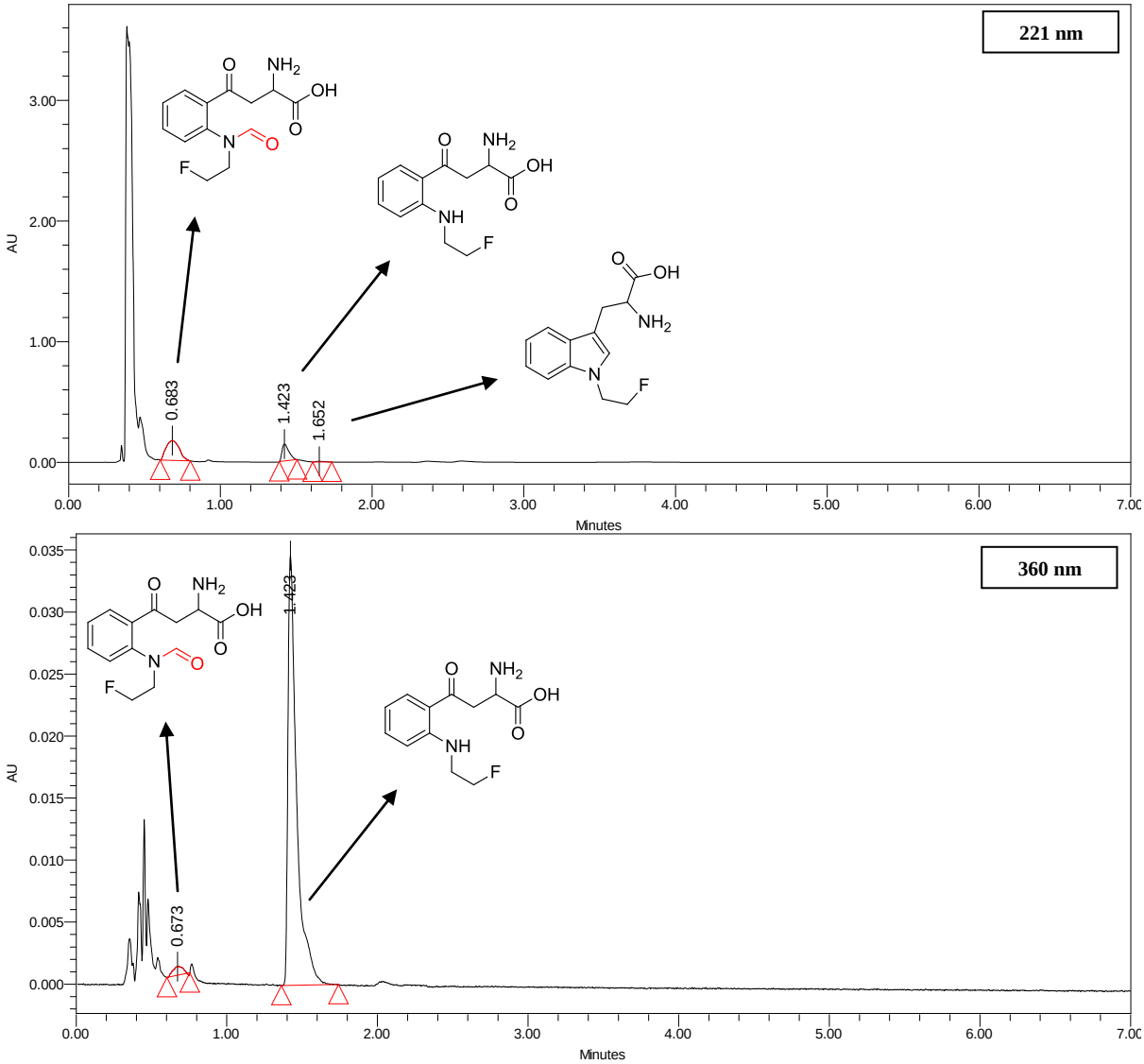
Retention time: 1.65 min **0.68 min** **1.42 min**

<u>Remarque:</u> The *N*-formyl-kynurenine derivative has a formyl groupment (in red), less easily cleavable in acidic media. This compound is thus observable in UPLC spectra.

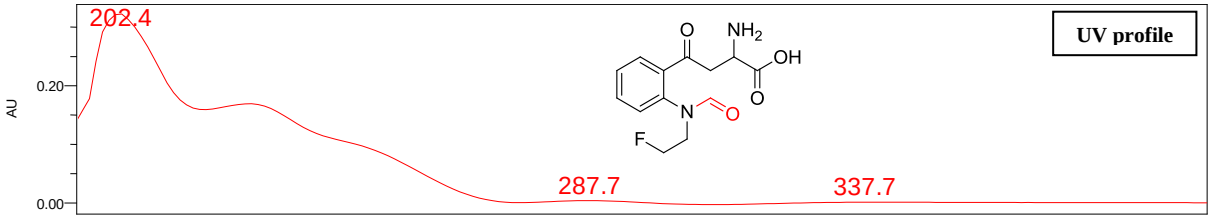
UPLC spectrum of 1-(2-fluoroethyl)-tryptophan reference:

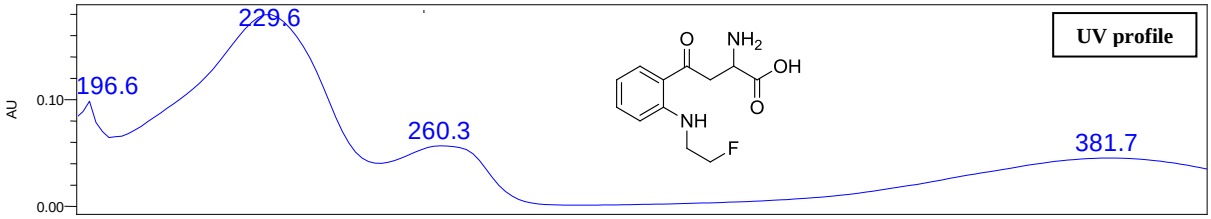


UPLC spectrum obtained after *in vitro* enzymatic assays with 1-(2-fluoroethyl)-tryptophan and rhIDO followed by hydrolysis with perchloric acid:

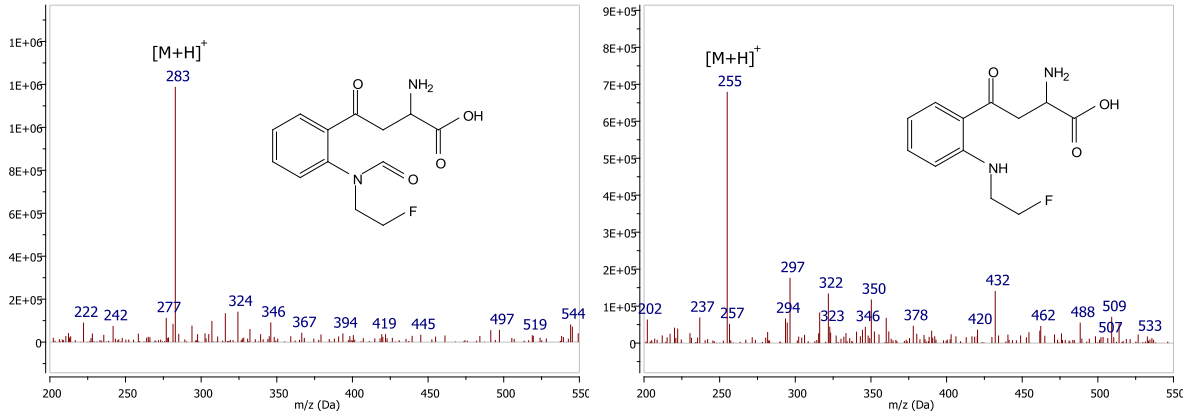


UV profile of integrated peaks

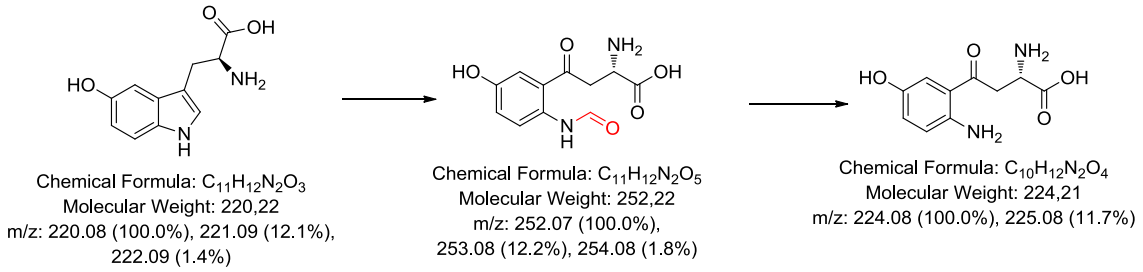




Mass Spectra of the collected peak obtained at 0.68 min and 1.42 min respectively



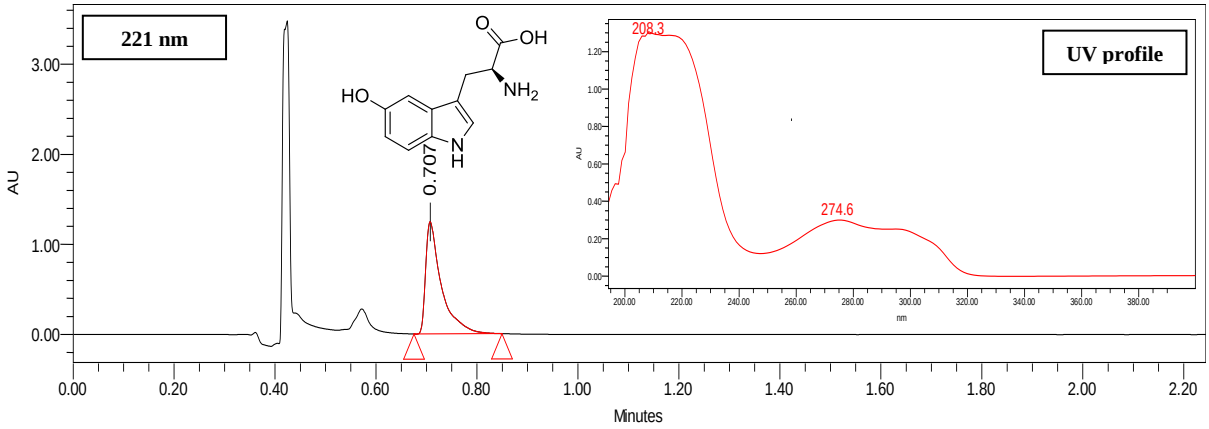
d. 5-HO-L-Trp (5-hydroxy-L- tryptophan) (13):



Retention time: 0.71 min – **0.55 min**

<u>Remarque:</u> The *N*-formyl-kynurenine derivative has a formyl groupment (in red), easily cleavable in acidic media. This compound is thus not observable in UPLC spectra.

UPLC spectrum of 5-HO-L-tryptophan reference:



UPLC spectrum obtained after *in vitro* enzymatic assays with 5-HO-L-tryptophan and rhIDO followed by hydrolysis with perchloric acid: