Supporting information

Synthesis and preclinical evaluation of 6-[^{18}F]Fluorine-Alpha-Methyl-L-Tryptophan, a novel PET tracer for measuring tryptophan uptake.

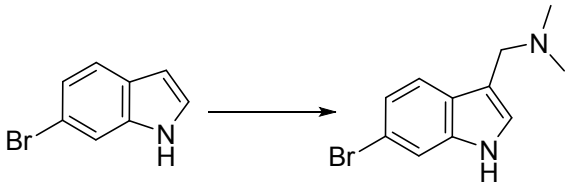
Raisa Krasikova†ł, Mikhail Kondrashov†, Camilla Avagliano†, Mikhail Petukhov¶#, Ana Vazquez-Romero†, Evgeny Revunov†, Peter Johnström†‡, Lenke Tari†, Miklós Tóth†, Jenny Häggkvist†, Sophie Erhardt§, Simon Cervenka† and Magnus Schou†‡*

†Centre for Psychiatry Research, Department of Clinical Neuroscience, Karolinska Institutet, & Health Care services, Region Stockholm, SE-171 76, Stockholm, Sweden
¶ Petersburg Nuclear Physics Institute named after B.P. Konstantinov, NRC "Kurchatov Institute", Gatchina, Russia
# Russian Scientific Center of Radiology and Surgical Technologies named after A.M. Granov, St. Petersburg, Russia
§Department of Physiology & Pharmacology, Karolinska Institutet, SE- 171 77 Stockholm, Sweden
‡PET Science Centre, Precision Medicine and Genomics, IMED Biotech Unit, AstraZeneca, Karolinska Institutet, S-171 76 Stockholm, Sweden
łN.P. Bechtereva Institute of Human Brain, Russian Academy of Science, 9, Pavlov str., 197376, St. Petersburg, Russia.

Contents

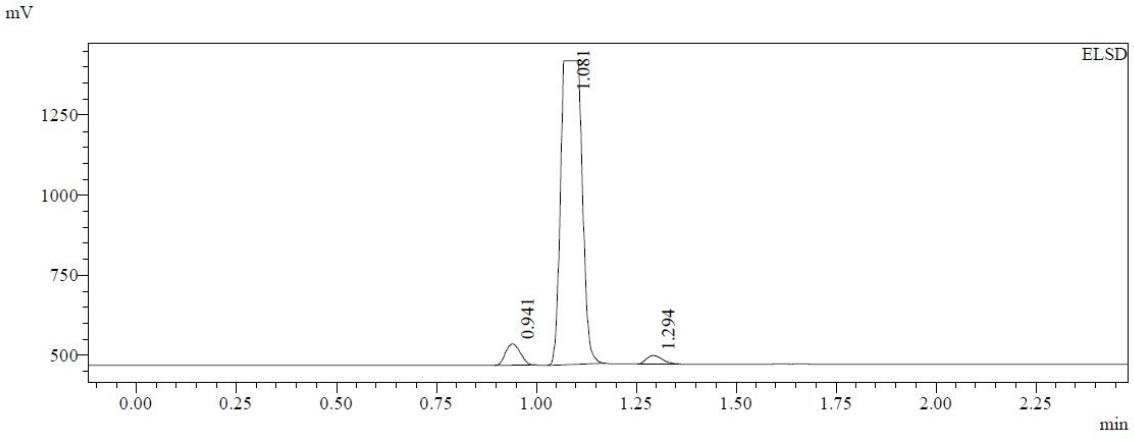
Synthesis of precursor material

[(6-Bromo-1H-indol-3-yl)methyl]dimethylamine (2)



Into a 250-mL round-bottom flask, was placed 6-bromo-indole (**1**) (21.6 g, 110.34 mmol, 1.00 equiv), acetic acid (40 mL), formaldehyde aqueous solution (37%, w/w)(9.93 g, 122.47 mmol, 1.10 equiv), dimethylamine aqueous solution (33%, w/w) (16.7 g, 122.47 mmol, 1.10 equiv) was added at 0°C in an ice/water bath. The resulting solution was allowed to stir for an additional 16 h at 25°C. The pH value of the solution was adjusted to 12 with sodium hydroxide aqueous solution. The resulting mixture was extracted with 3x500 mL of chloroform and the organic layers were combined, dried over anhydrous sodium sulfate and concentrated under vacuum. This resulted in 18 g (65%) of [(6-bromo-1H-indol-3-yl)methyl]dimethylamine as a brown solid.

LC-MS: (ES, *m/z*): $[M+1]^+$ = 253, 255, T=1.08 min

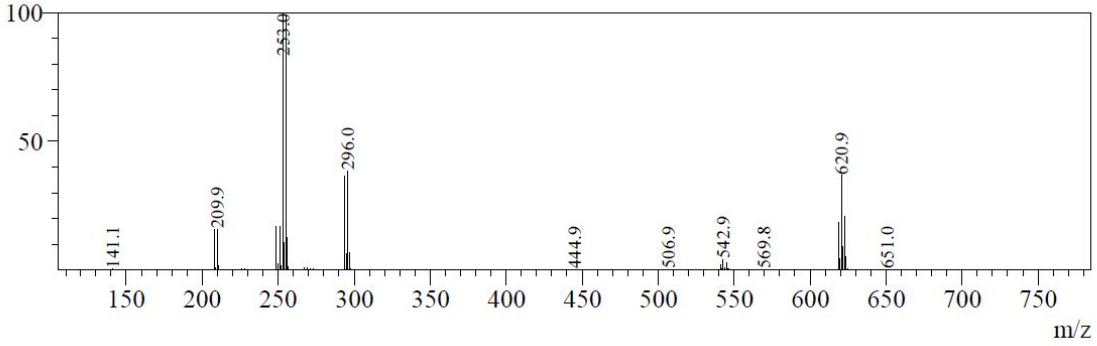


ELSD

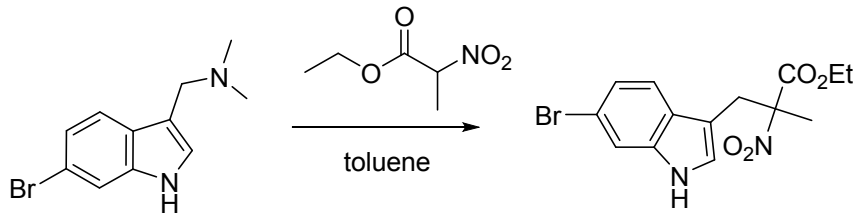
Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.941	65115	6.259	170842	4.592
2	1.081	949257	91.243	3479600	93.528
3	1.294	25984	2.498	69949	1.880
Total				3720390	100.000

Mass Spectrum

Line#:1 R.Time:1.085(Scan#:169)
MassPeaks:94
Spectrum Mode:Averaged 1.078-1.092(168-170) Base Peak:253.0(897794)
BG Mode:Calc Segment 1 - Event 1

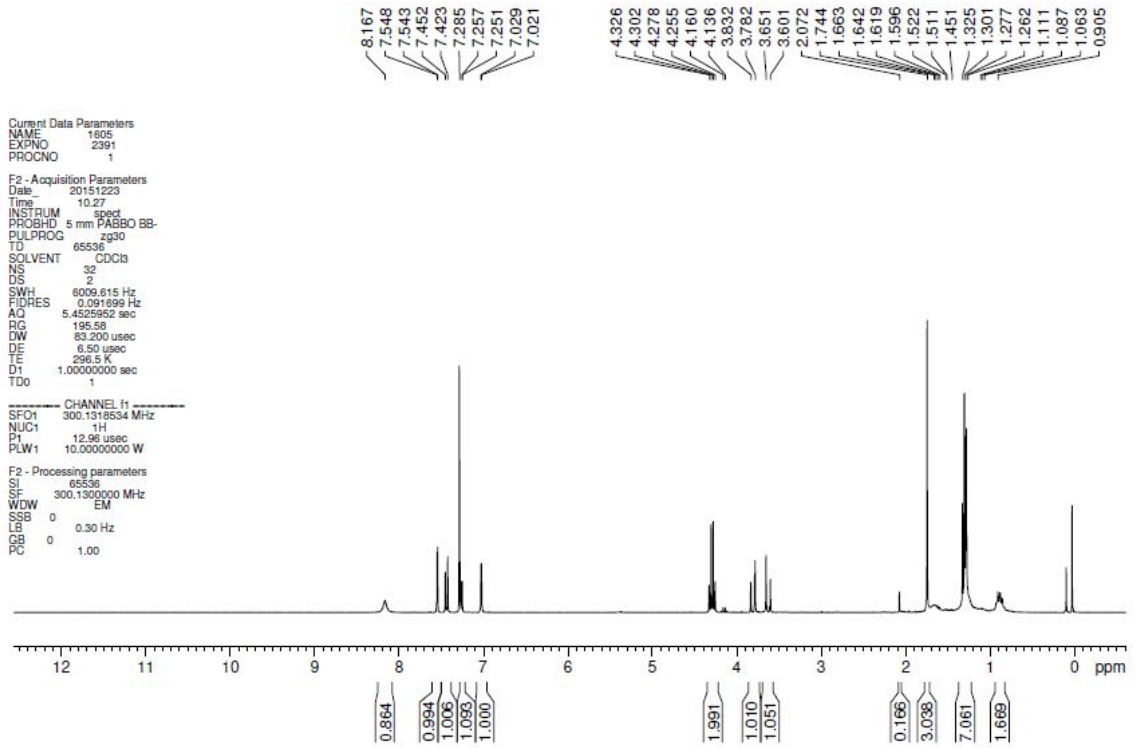


Ethyl 3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (Rac-3)

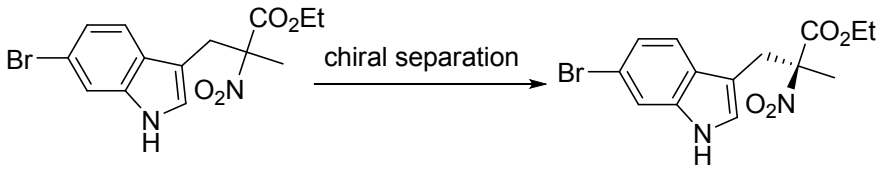


Into a 250-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed [(6-bromo-1H-indol-3-yl)methyl]dimethylamine (**2**) (18 g, 71.42 mmol, 1.00 equiv), ethyl 2-nitropropanoate (12.60 g, 85.70 mmol, 1.20 equiv), toluene (120 mL). The resulting solution was stirred for 24 h at 105°C. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1/5). This resulted in 6 g (24%) of ethyl 3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a yellow solid.

^{1}H-NMR (300 MHz, CDCl3, *ppm*): δ 8.18 (1H, s), 7.55-7.45 (1H, m), 7.45- 7.42 (1H, m), 7.29-7.25 (1H, m), 7.03-7.02 (1H, m), 4.33-4.16 (2H, m), 3.83-3.60(2H, m), 1.74 (3H, s), 1.33-1.26 (3H, m).

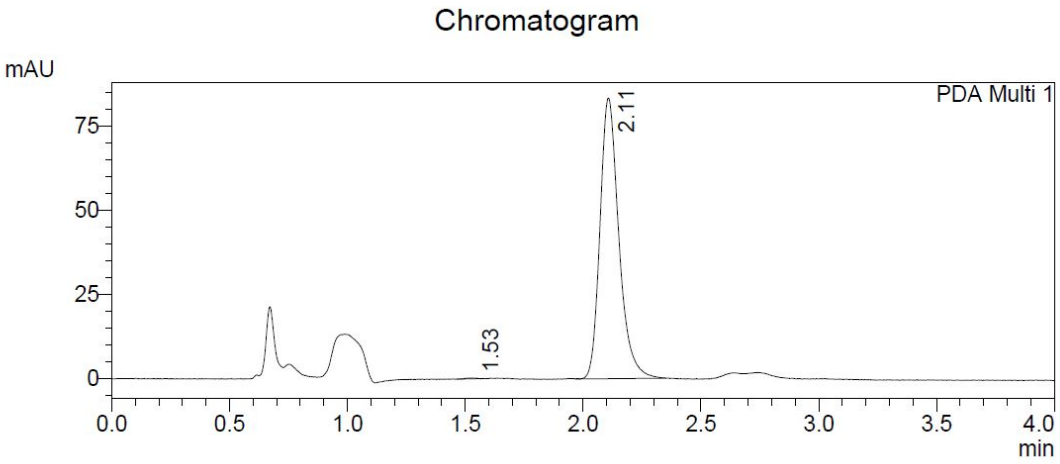


Ethyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (3)



The wanted isomer was separated by Chiral-Prep-HPLC with the following conditions: Column, DAICEL CHIRALPAK AD-H; mobile phase: ethanol/hexane=20/80.This resulted in 2 g (33%) of ethyl (R)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a yellow solid and 2g (35%) of methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a yellow solid.

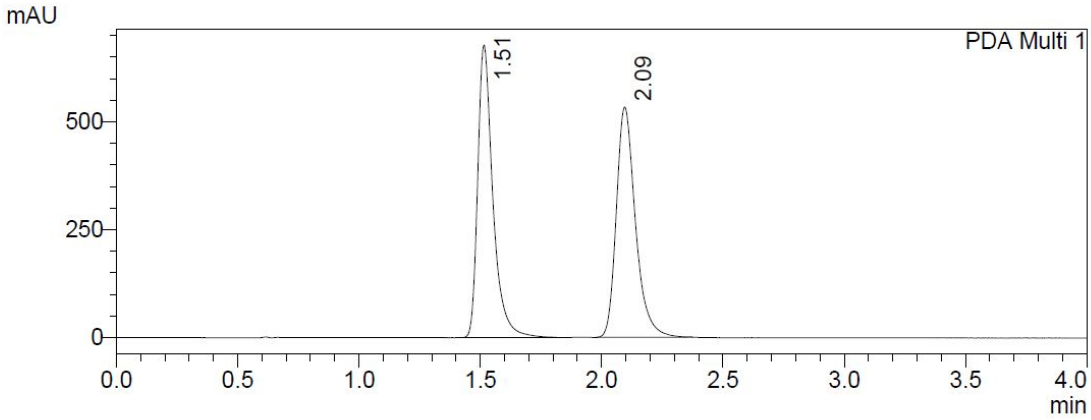
CHIRAL-HPLC; T=2.11 min



PeakTable

PDA Ch1 220nm 4nm

Peak#	Ret. Time	Height	Area	Area %	Width(50%)	Resolution
1	1.531	214	815	0.18	0.062	0.000
2	2.107	83094	462258	99.82	0.083	4.901
Total			463073	100.00		

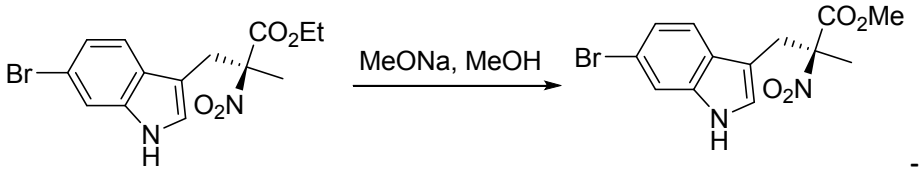


PeakTable

PDA Ch1 220nm 4nm

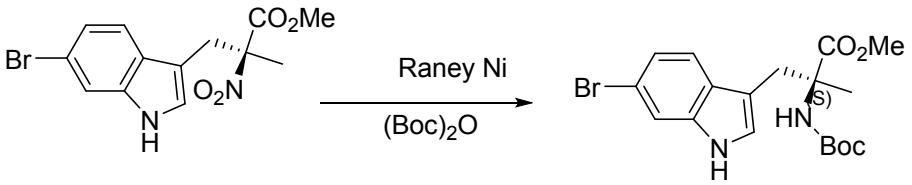
Peak#	Ret. Time	Height	Area	Area %	Width(50%)	Resolution
1	1.515	673644	2941246	50.15	0.063	0.000
2	2.094	531225	2923567	49.85	0.082	4.694
Total			5864813	100.00		

Methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (4)



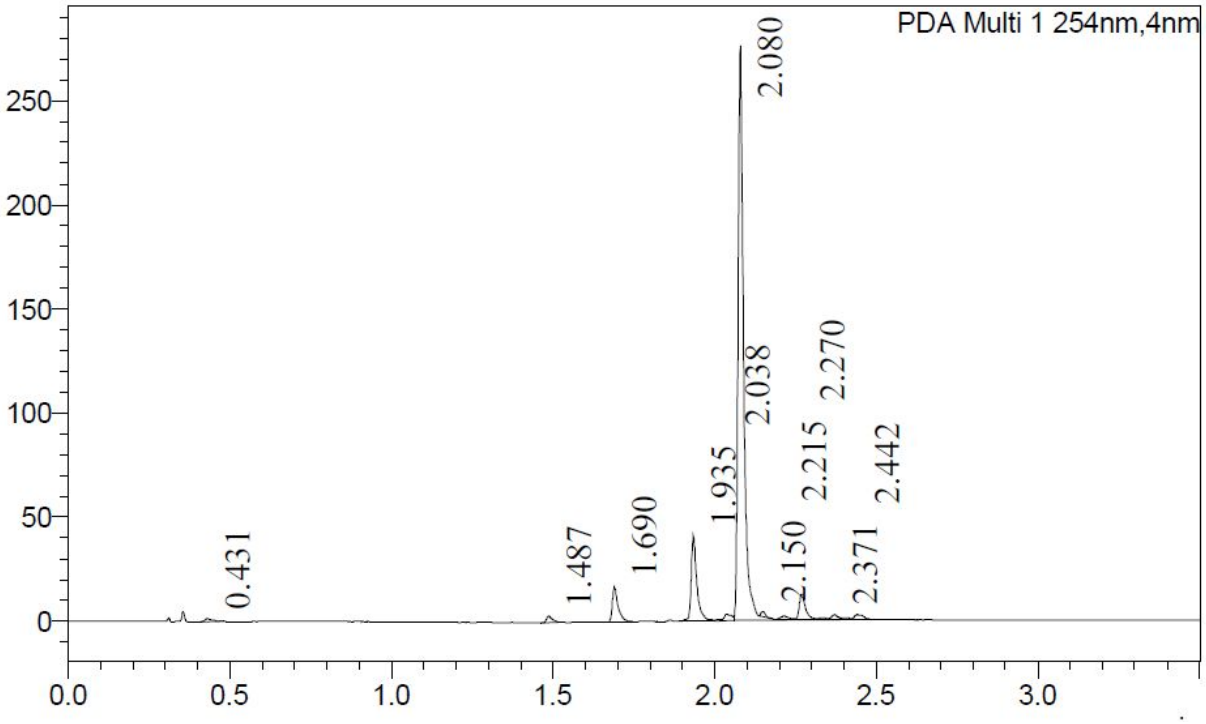
Into a 25-mL round-bottom flask, was placed ethyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (**3**) (1.8 g, 5.07 mmol, 1.00 equiv), sodium methoxide (540 mg, 10.00 mmol, 2.00 equiv), methanol (10 mL). The resulting solution was stirred for 2 h at 25°C.The resulting solution was diluted with 20 mL of water, extracted with 3x200 mL of ethyl acetate and the organic layers were combined and evaporated. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1/1). This resulted in 1.3 g (75%) of methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a yellow solid.

Methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-[[(tert-butoxy)carbonyl]amino]-2-methylpropanoate (5)

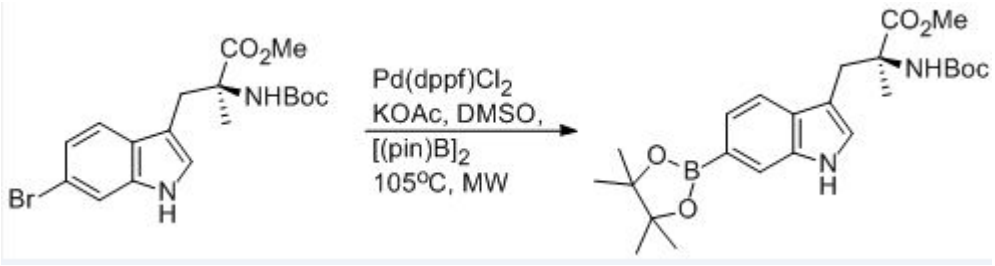


Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (**4**) (1.2 g, 3.52 mmol, 1.00 equiv), di-tert-butyl dicarbonate (3.8 g, 17.41 mmol, 5.00 equiv), methanol (25 mL), Raney Ni (600 mg, slurry in water). The flask was evacuated and flushed three times with nitrogen, followed by flushing with hydrogen. The mixture was stirred 2 h at room temperature under an atmosphere of hydrogen (balloon, almost 1 atm H_2). The solids were filtered out. The resulting mixture was concentrated under vacuum. The resulting solution was extracted with 3x100 mL of ethyl acetate and the organic layers combined and concentrated under vacuum. This resulted in 0.8 g (55%) of methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-[[(tert-butoxy)carbonyl]amino]-2-methylpropanoate as a yellow solid.

LC-MS: (ES, *m/z*): $[M+1]^+$ = 410, T=2.08 min

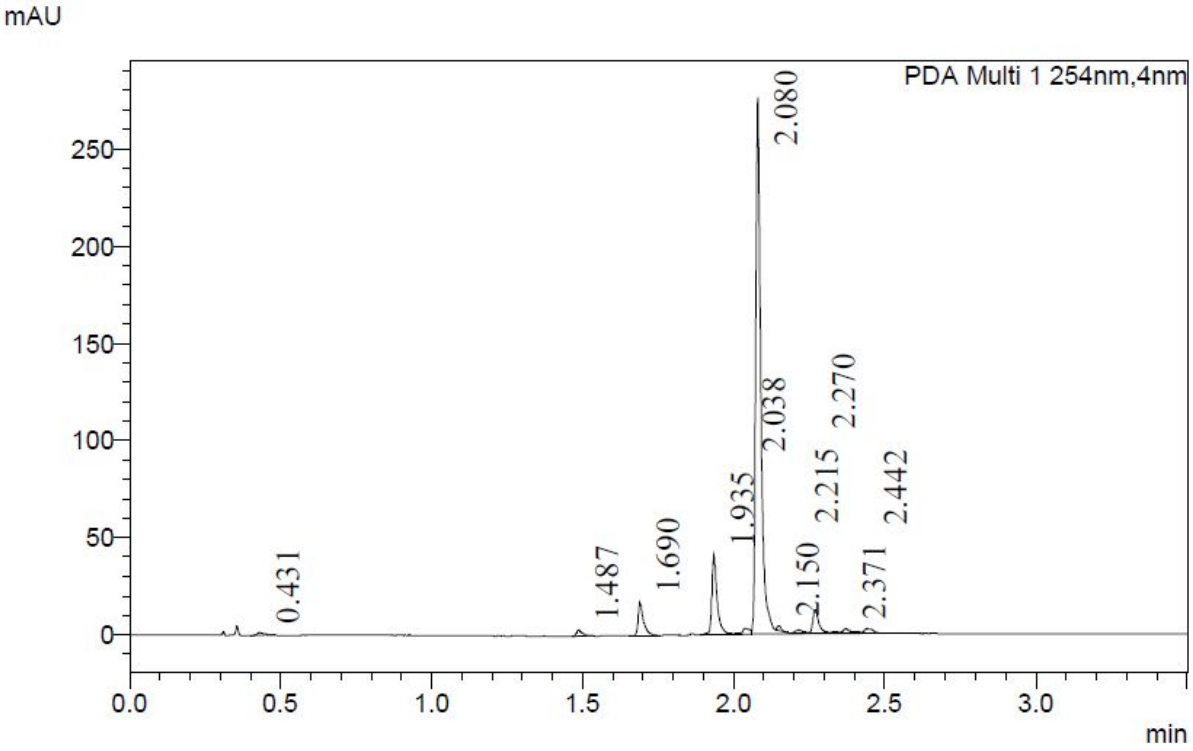

PDA Multi 1 254nm,4nm
250
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0.431
1.487
1.690
1.935
2.038
2.080
2.150
2.215
2.270
2.371
2.442

Methyl (S)-2-[[(tert-butoxy)carbonyl]amino]-2-methyl-3-[6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl]propanoate (6)

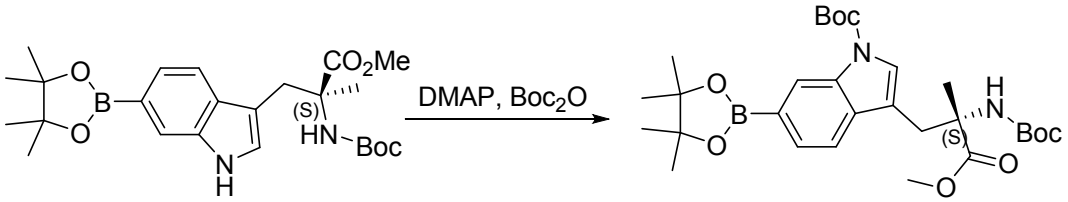


Into a 20-mL vial, was placed methyl (S)-3-(6-bromo-1H-indol-3-yl)-2-[[(tert-butoxy)carbonyl]amino]-2-methylpropanoate (**5**) (800 mg, 1.95 mmol, 1.00 equiv), CH_3COOK (573 mg, 5.85 mmol, 3.00 equiv), 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.98 g, 7.80 mmol, 4.00 equiv), $Pd(dppf)Cl_2$ (163 mg, 0.20 mmol, 0.10 equiv), ethylene glycol dimethyl ether (10 mL). The final reaction mixture was irradiated with microwave radiation for 30 min at 105°C. The resulting solution was extracted with 3x100 mL of ethyl acetate and the organic layers combined and dried over anhydrous sodium sulfate. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1/4). This resulted in 400 mg (45%) of methyl (S)-2-[[(tert-butoxy)carbonyl]amino]-2-methyl-3-[6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl]propanoate as a white solid.

LC-MS: (ES, *m/z*): $[M+1]^+$ = 459, T=2.11 min



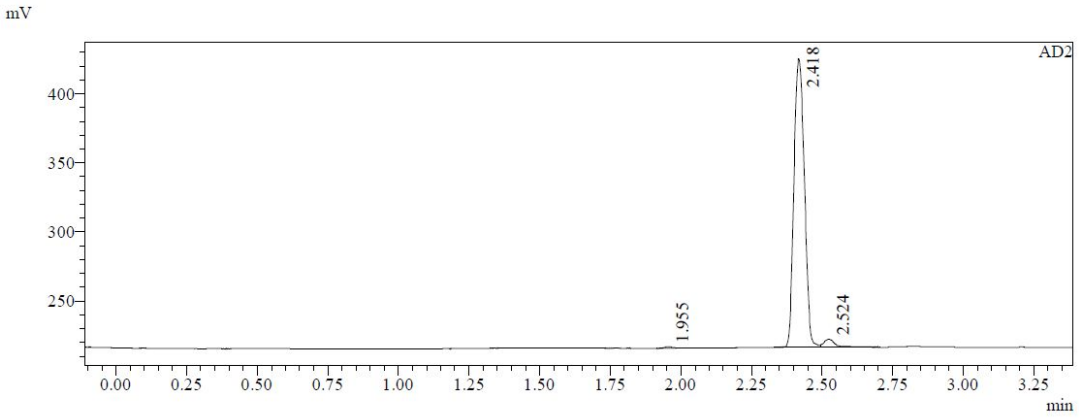
***tert*-Butyl 3-[(S)-2-[[(tert-butoxy)carbonyl]amino]-3-methoxy-2-methyl-3-oxopropyl]-6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-1-carboxylate (7)**



Into a 25-mL round-bottom flask, was placed methyl (S)-2-[[(*tert*-butoxy)carbonyl]amino]-2-methyl-3-[6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl]propanoate (**6**) (400 mg, 0.87 mmol, 1.00 equiv), TEA (176 mg, 1.74 mmol, 2.00 equiv), DMAP (20.7 mg, 0.17 mmol, 0.2 equiv), MeCN (10 mL), di-tert-butyl dicarbonate (571 mg, 2.62 mmol, 3.00 equiv). The resulting solution was stirred for 2 h at 25^{o}C. The reaction was then quenched by the addition of 20 mL of water. The resulting solution was extracted with 3x50 mL of ethyl acetate and the organic layers were combined and evaporated. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1/5). This resulted in 200 mg (41%) of tert-butyl 3-[(S)-2-[[(tert-butoxy)carbonyl]amino]-3-methoxy-2-methyl-3-oxopropyl]-6-(tetramethyl-1,3,2-dioxaborolan-2-yl)-indole-1-carboxylate as a white solid.

LC-MS: (ES, *m/z*): $[M+1]^+$ = 559, T =2.42 min

ELS Chromatogram

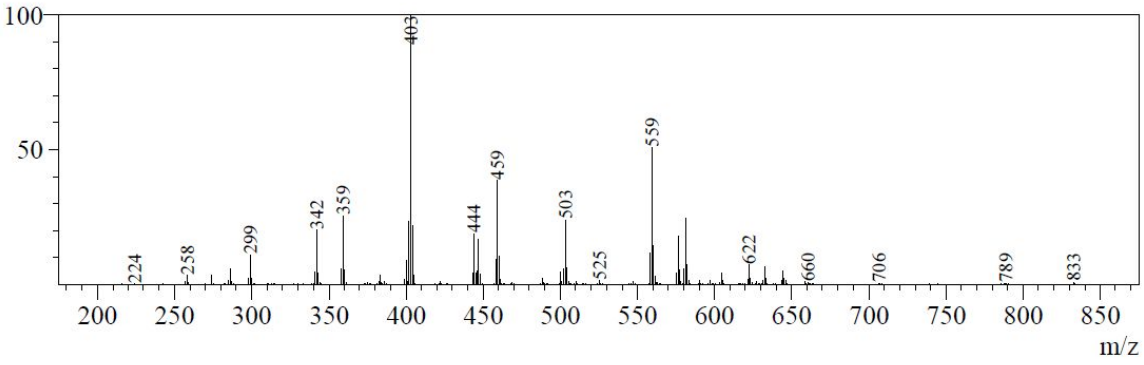


AD2

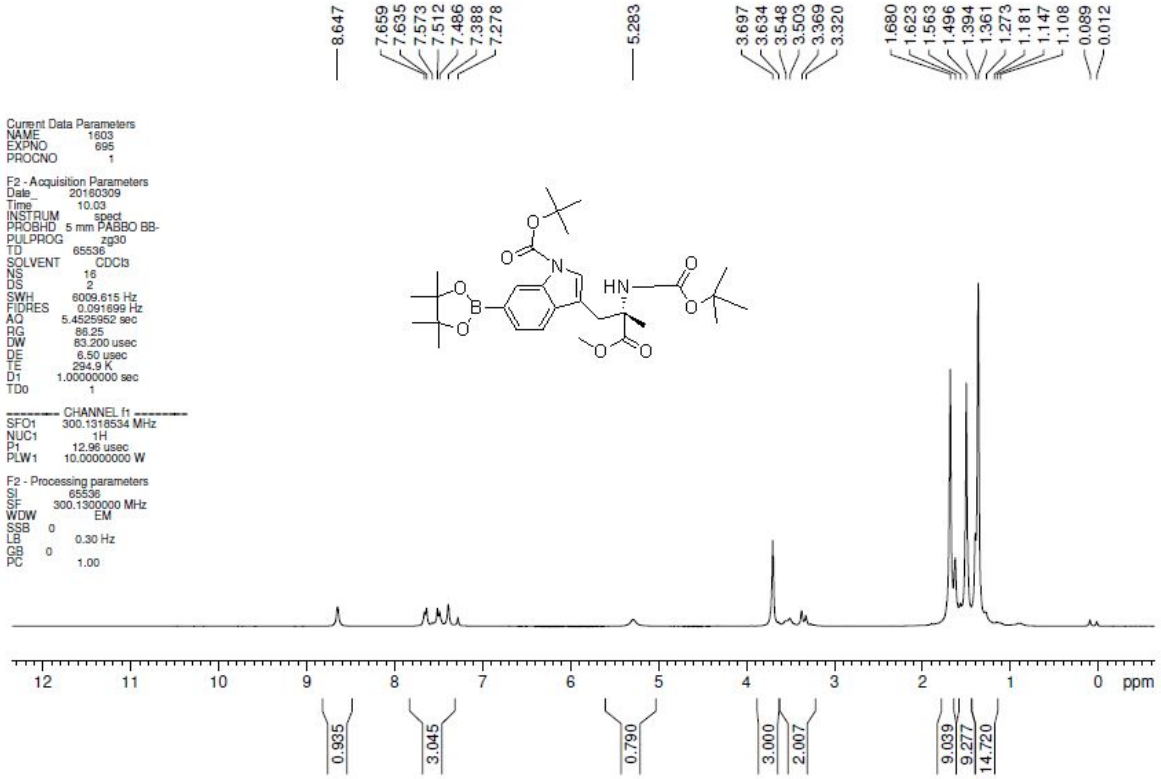
Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.955	917	0.429	2322	0.416
2	2.418	207182	96.923	539896	96.727
3	2.524	5661	2.648	15947	2.857
Total				558165	100.000

Mass Spectrum

Line#:1 R.Time:2.417(Scan#:368)
MassPeaks:312
Spectrum Mode:Averaged 2.410-2.423(367-369) Base Peak:403(1359695)
BG Mode:Calc Segment 1 - Event 1

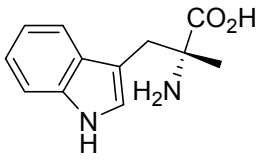


^{1}H-NMR-PH-AZA-221-AZ15-053-B-0: (CDCl3, *ppm*): 8.65 (1H, s), 7.66-7.28 (3H, m), 5.28 (1H, s), 3.68 (3H, s), 3.63-3.32 (2H, m), 1.68-1.50 (9H, m), 1.39-1.36 (9H, m), 1.27-1.11 (15H, m).



Confirmation of the stereochemical identity

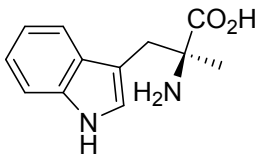
In order to confirm the R/S configuration, we synthesized both enantiomers of 2-amino-3-(1H-indol-3-yl)-2-methylpropanoic acid, tested the specific rotation for both and finally compared with the value that reported in references.



(R)-4

reported optical rotation value:
16 ^{o} (in methanol, 589 nm)

20 mg from the first eluent
optical rotation (+): 17.6 ^{o} (in methanol, 589 nm)



(S)-4

reported optical rotation value:
-11.3 ^{o} (in H_2O, 589 nm)

50 mg from the second eluent
optical rotation (-):-12.6 ^{o} (in H_2O, 589 nm)

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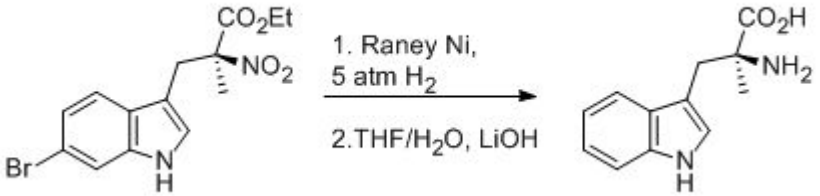
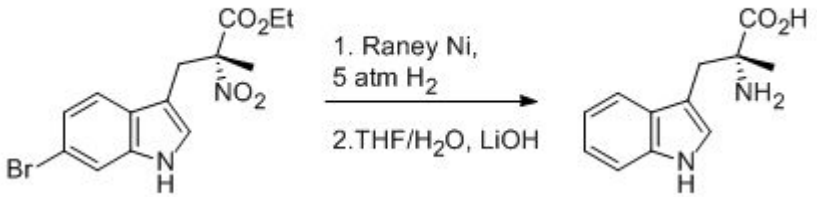


Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed Raney Ni (50 mg, slurry in water) in anhydrous MeOH (5 mL), then ethyl (S)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (100 mg, 0.28 mmol, 1.00 equiv) was added to the solution. The flask was evacuated and flushed three times with hydrogen and stirred for 2 hours at room temperature under 5 atm H_2. The mixture was filtrated and the filtrate was concentrated under vacuum. The residue was dissolved in tetrahydrofuran (2 mL) and water (0.5 mL) before LiOH (15 mg, 0.63 mmol, 2.00 equiv) was added. The solution was stirred for another 2 h at room temperature. The mixture was concentrated under vacuum and purified by C18 column to get 50 mg (60%) of (S)-2-amino-3-(6-bromo-1H-indol-3-yl)-2-methylpropanoic acid as a white solid.

LC-MS-PH-AZA-221-AZ15-053-B-19: (ES, *m/z*): $[M+1]^+$ = 219, T=0.93min



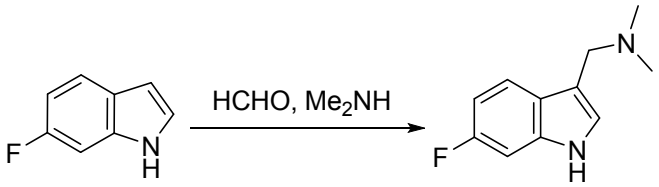
Into a 25-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed Raney Ni (50 mg, crude, slurry in water) in anhydrous MeOH (5 mL), then ethyl (R)-3-(6-bromo-1H-indol-3-yl)-2-methyl-2-nitropropanoate (100 mg, 0.28 mmol, 1.00 equiv) was added to the solution. The flask was evacuated and flushed three times with hydrogen and stirred for 2 hours at room temperature under 5 atm H_2. The mixture was filtrated and the filtrate was concentrated under vacuum. The residue was dissolved in tetrahydrofuran (2 mL) and water (0.5 mL) before LiOH (15 mg, 0.63 mmol, 2.00 equiv) was added. The solution was stirred for another 2 h at room

temperature. The mixture was concentrated under vacuum and then purified by C18 column to get 50 mg (50%) of (R)-2-amino-3-(6-bromo-1H-indol-3-yl)-2-methylpropanoic acid as a white solid.

LC-MS-PH-AZA-221-AZ15-053-B-18: (ES, *m/z*): $[M+1]^+$ = 219, T=0.93 min

Synthesis of unlabeled reference material

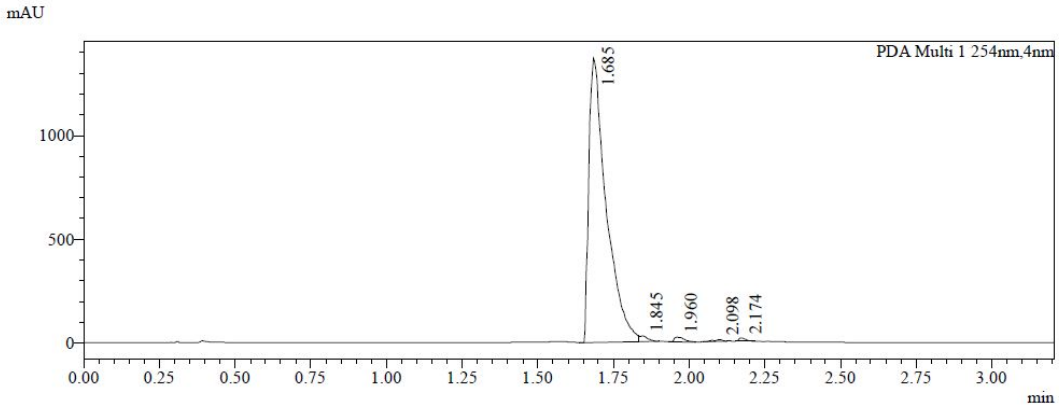
[(6-Fluoro-1H-indol-3-yl)methyl]dimethylamine (9)



Into a 50-mL round-bottom flask, was placed acetic acid (5 mL), formaldehyde aqueous solution (37%, w/w) (1.2 g, 14.8 mmol, 1.06 equiv), dimethylamine aqueous solution (33%, w/w) (2.09 g, 15.30 mmol, 1.1 equiv), 6-fluoro-indole (**8**) (1.88 g, 13.91 mmol, 1.0 equiv) at 0°C. The resulting solution was stirred for 16 h at 25°C. The pH value of the solution was adjusted to 12 with NaOH aqueous solution (3 M). The resulting solution was extracted with 3x100 mL of dichloromethane and the organic layers combined and concentrated under vacuum. This resulted in 2 g (75%) of [(6-fluoro-1H-indol-3-yl)methyl]dimethylamine as a yellow solid.

LC-MS: (ES, *m/z*): $[M+1]^+$ = 193; T=1.69min

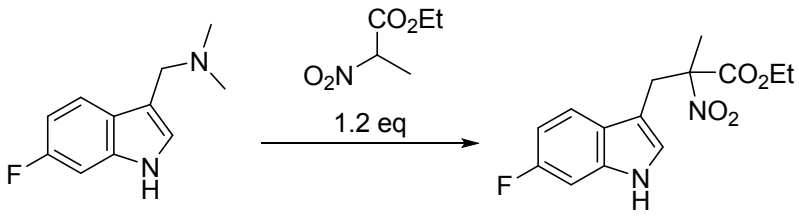
PDA Chromatogram



PDA Ch1 254nm

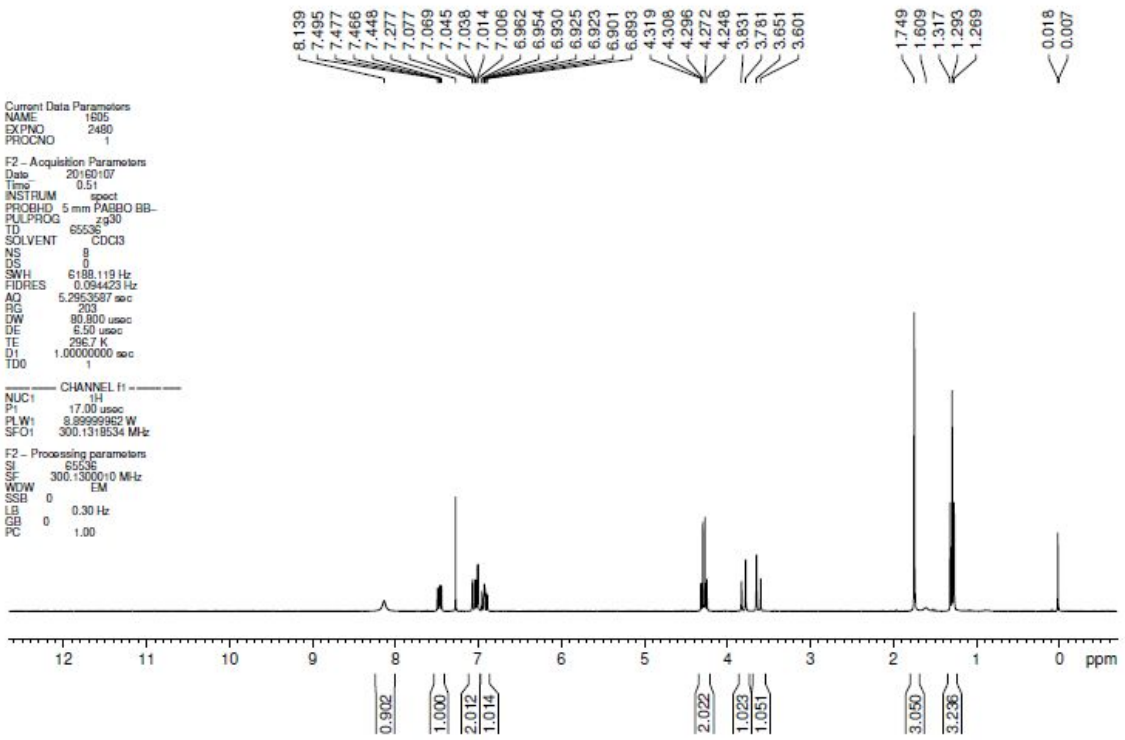
Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.685	1371191	94.838	5464563	97.432
2	1.845	26899	1.860	50568	0.902
3	1.960	22760	1.574	48857	0.871
4	2.098	8860	0.613	16639	0.297
5	2.174	16122	1.115	27966	0.499
Total				5608592	100.000

Ethyl 3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate (Rac-10)



Into a 100-mL round-bottom flask, was placed ethyl 2-nitropropanoate (1.73 g, 11.76 mmol, 1.20 equiv), [(6-fluoro-1H-indol-3-yl)methyl]dimethylamine (**8**) (1.87 g, 9.73 mmol, 1.00 equiv), toluene (30 mL). The resulting solution was stirred for 24 h at 105°C in an oil bath. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column and eluting with ethyl acetate/petroleum ether (1:5) to get 1.2 g (42%) of ethyl 3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a yellow solid.

H-NMR (300 MHz, CDCl3, *ppm*): δ 8.14 (1H, s), 7.50-7.45 (1H, m), 7.08-7.01 (2H, m), 6.96-6.89 (1H, m), 4.32-4.25 (2H, m), 3.72 (2 H, dd, *J1* = 54 Hz, *J2* = 15 Hz), 1.75(3H, s), 1.32-1.27 (3H, m).


8.139
7.495
7.477
7.466
7.448
7.277
7.077
7.069
7.045
7.038
7.014
7.006
6.962
6.954
6.930
6.925
6.923
6.901
6.893
4.319
4.308
4.296
4.272
4.248
3.831
3.781
3.651
3.601
1.749
1.609
1.317
1.293
1.269
0.018
0.007
Current Data Parameters
NAME 1605
EXPNO 2480
PROCNO 1
F2 – Acquisition Parameters
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Time 0.51
INSTRUM spect
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PULPROG zg30
TD 65536
SOLVENT CDCl3
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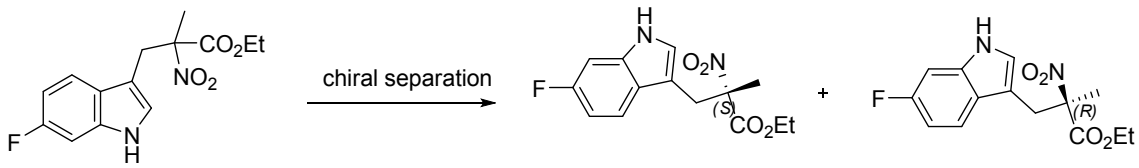
DS 0
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 203
DW 80.800 usec
DE 6.50 usec
TE 296.7 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 17.00 usec
PLW1 8.89999962 W
SFO1 300.1319534 MHz
F2 – Processing parameters
SI 65536
SF 300.1300010 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.902
1.000
2.012
1.014
2.022
1.023
1.051
3.050
3.236

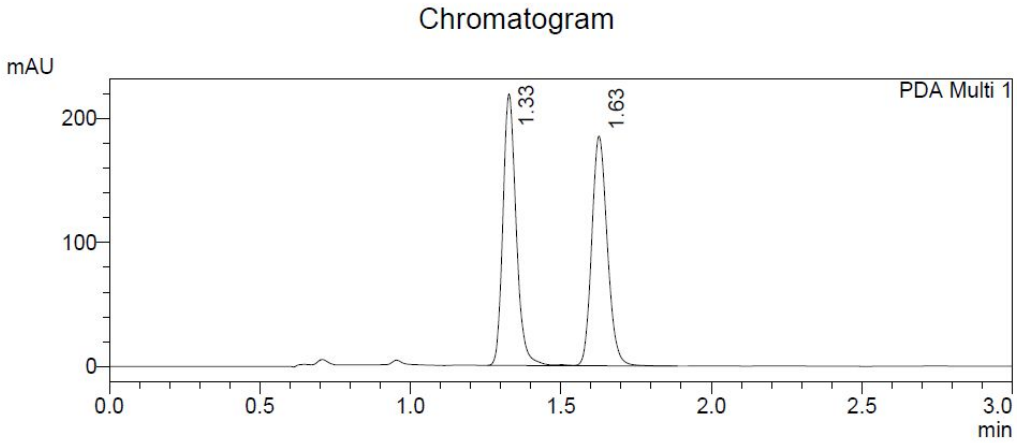
(*S*)-3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate (10)



The two isomers was separated by Chiral-Prep-HPLC with the following conditions: Column, DAICELPAK AD-H; mobile phase,: n-hexane(0.001MIPA)/Ethanol=80/20). This resulted in 500 mg (42%) of ethyl (R) 3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a white solid and 500 mg (42%) of ethyl ((S)-3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate as a white solid.

CHIRAL-HPLC, R-isomer: Tr=1.33min

CHIRAL-HPLC, S-isomer: Tr=1.63min

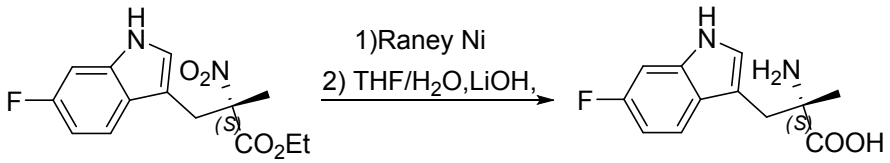


PeakTable

PDA Ch1 254nm 4nm

Peak#	Ret. Time	Height	Area	Area %	Width(50%)	Resolution
1	1.327	214744	673621	50.27	0.047	0.000
2	1.626	183986	666252	49.73	0.055	3.449
Total			1339874	100.00		

(S)-2-amino-3-(6-fluoro-1H-indol-3-yl)-2-methylpropanoic acid (11)

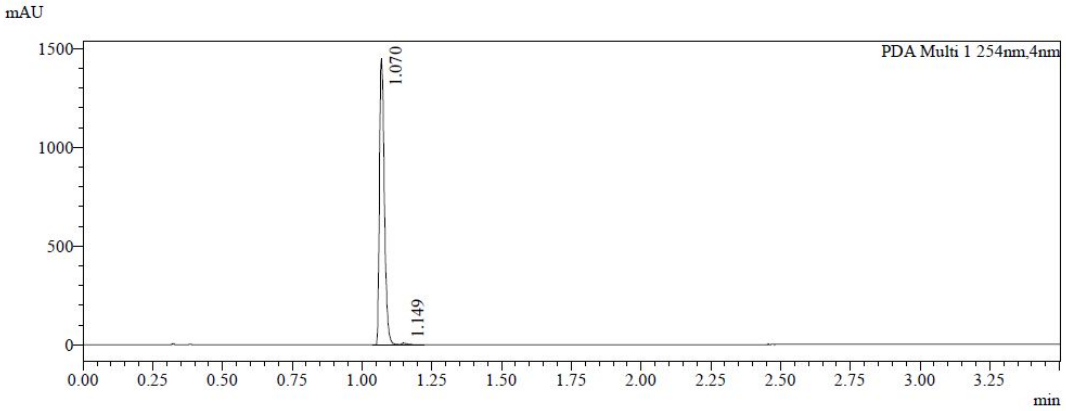


Into a 50-mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen, was placed Raney Ni (200 mg slurry in water) in anhydrous MeOH (20 ml), then assumed ethyl (S)-3-(6-fluoro-1H-indol-3-yl)-2-methyl-2-nitropropanoate (**10**) (350 mg, 1.19 mmol, 1.00 equiv) was added to the solution. The flask was evacuated and flushed three times with hydrogen and stirred for 2 hours at room temperature. The mixture was filtered and the filtrate was concentrated under vacuum. The residue was dissolved in tetrahydrofuran (4 ml) and water (1 ml) before LiOH (58 mg, 2.42 mmol, 2.0 equiv) was added. The solution was stirred for another 2 h at room temperature. The mixture was concentrated under vacuum The mixture was concentrated under vacuum and purified by prep HPLC to get 156 mg (56%) of (S)-2-amino-3-(6-fluoro-1H-indol-3-yl)-2-methylpropanoic acid as a yellow solid.

LC-MS (ES, *m/z*): $[M+1]^+$= 237, T=1.07min

H-NMR: (CD3OD, *ppm*): 7.66-7.61 (1H, m), 7.20 (1H, s), 7.08-7.04 (1H, m), 6.87-6.81 (1H, m), 3.56--3.09 (2H, m), 1.55 (3H, s).

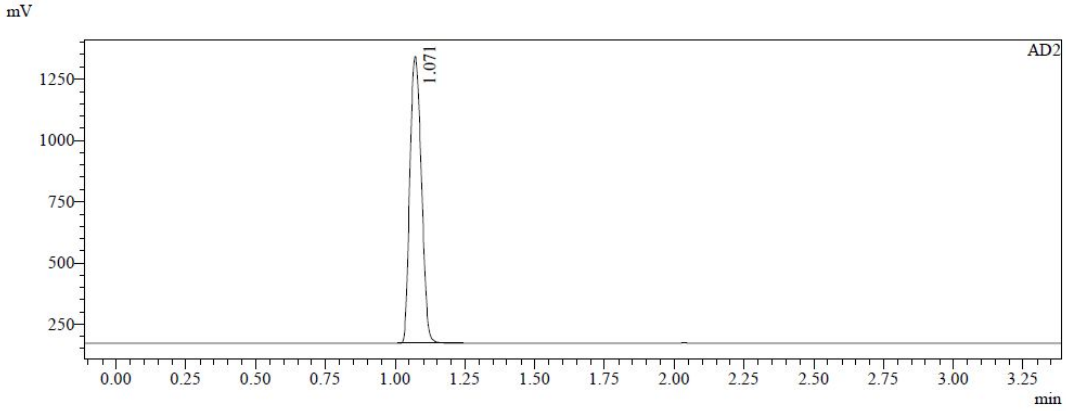
PDA Chromatogram



PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.070	1427733	99.436	1799526	99.406
2	1.149	8093	0.564	10754	0.594
Total				1810280	100.000

ELS Chromatogram

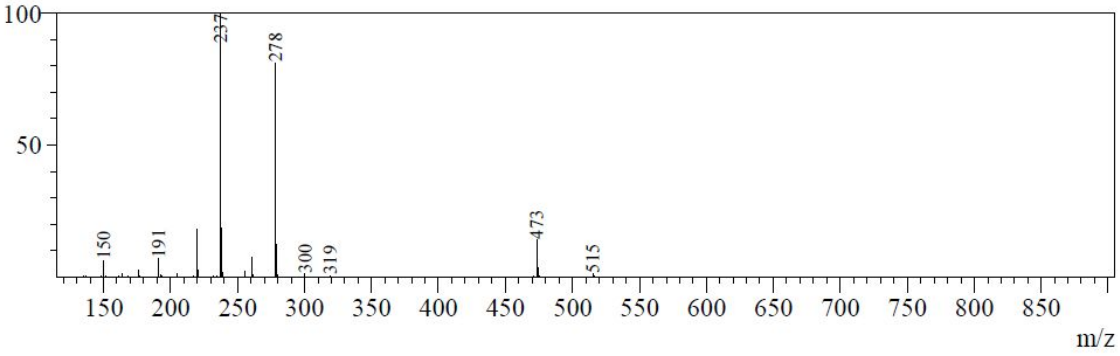


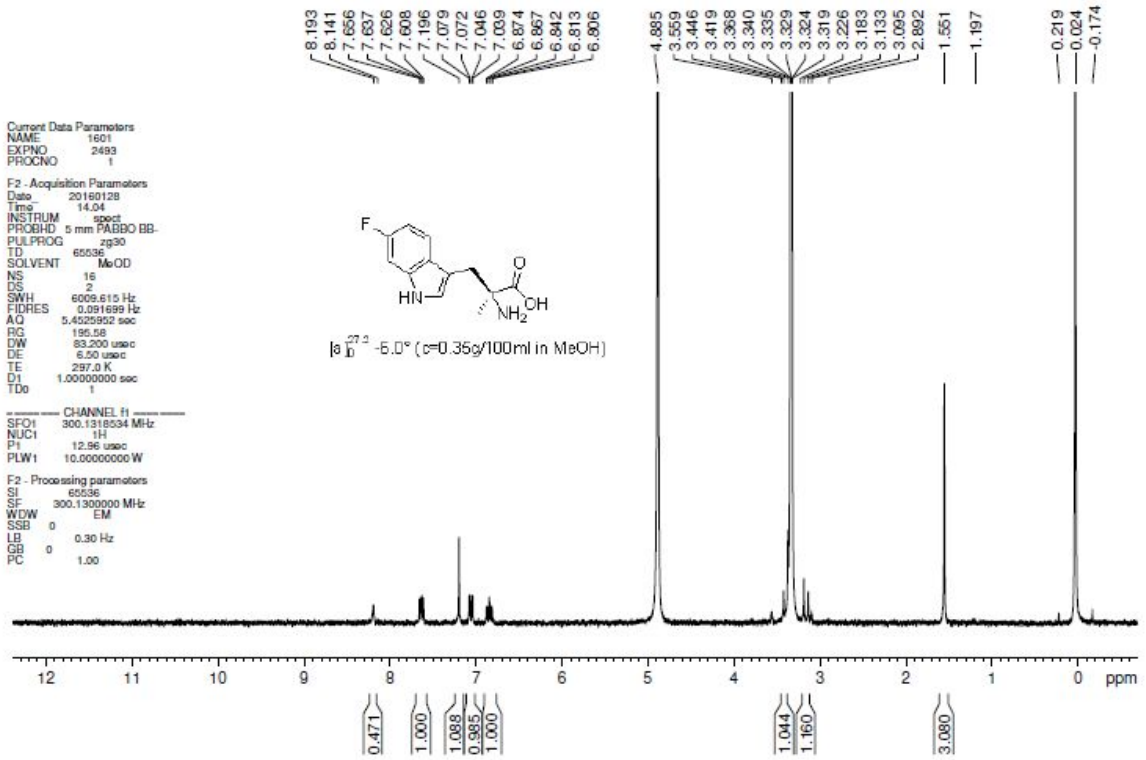
AD2

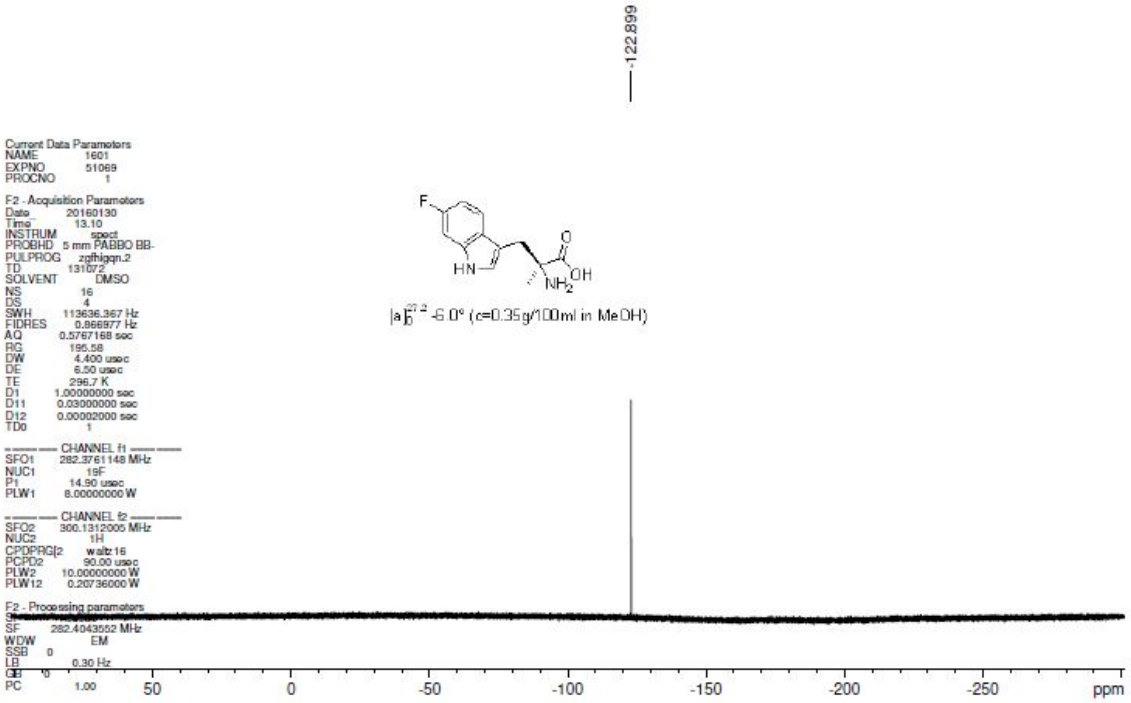
Peak#	Ret. Time	Height	Height%	Area	Area%
1	1.071	1157881	100.000	3375526	100.000
Total				3375526	100.000

Mass Spectrum

Line#:1 R.Time:1.077(Scan#:168)
MassPeaks:269
Spectrum Mode:Averaged 1.070-1.083(167-169) Base Peak:237(6316475)
BG Mode:Calc Segment 1 - Event 1



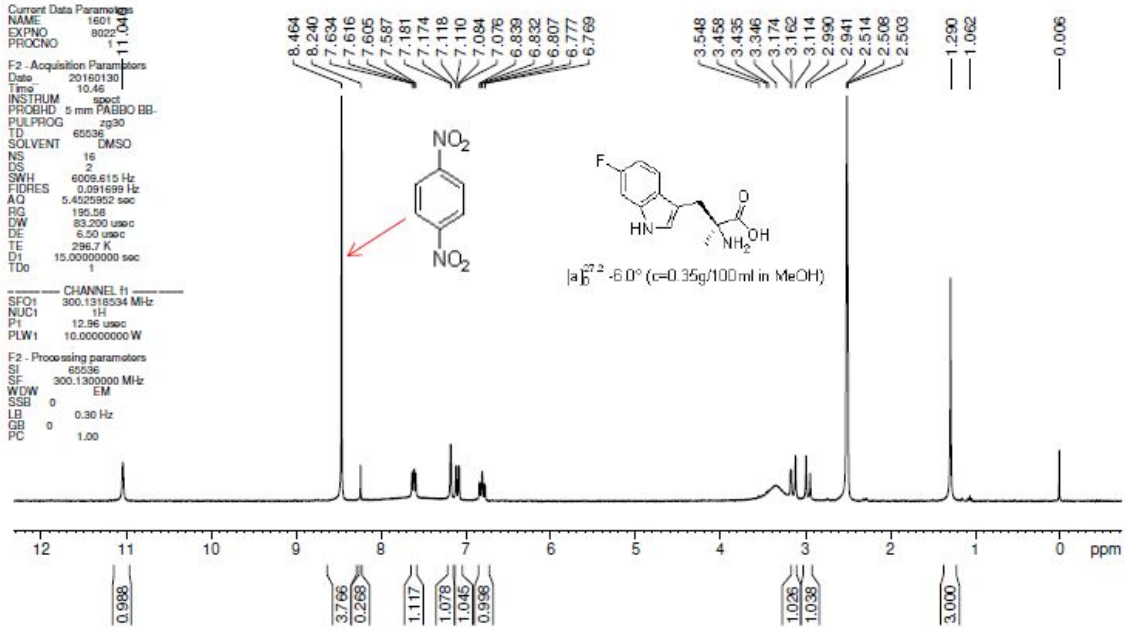




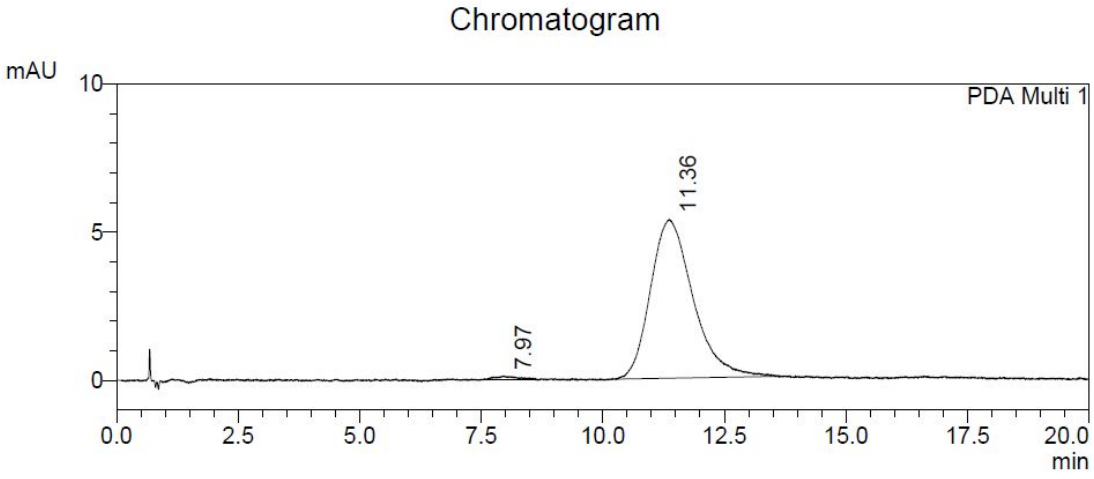
QNMR

S=1.54mg ; IS=1.03mg

IS 1H	Sam 1H	MW of SAM	MW of IS	Wt. IS	Wt. Sam	Intensity Sam	Intensity IS	Purity IS	Weight %
4	1	236	168	1.03	1.54	1	3.766	99.00	98.79



Chiral HPLC (pure (*S*)-isomer)

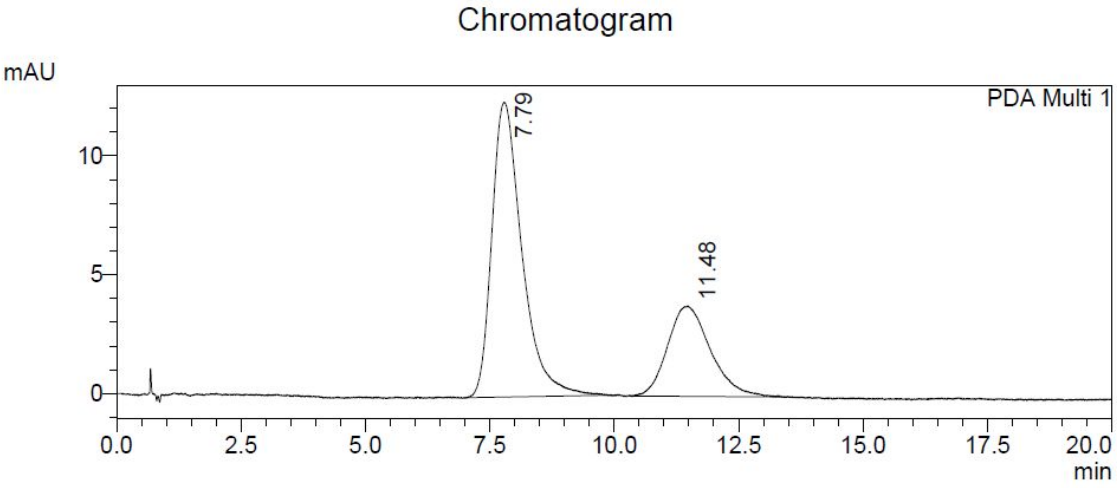


PeakTable

PDA Ch1 280nm 4nm

Peak#	Ret. Time	Height	Area	Area %	Width(50%)	Resolution
1	7.973	119	4061	1.23	0.351	0.000
2	11.364	5346	325173	98.77	0.915	4.040
Total			329234	100.00		

Chiral HPLC (mixture of (*R*)- and (S)-enantiomer



PeakTable

PDA Ch1 280nm 4nm

Peak#	Ret. Time	Height	Area	Area %	Width(50%)	Resolution
1	7.794	12387	521736	69.67	0.618	0.000
2	11.483	3795	227161	30.33	0.902	2.759
Total			748897	100.00		

Enzyme assays

High performance liquid chromatography (HPLC)

All HPLC analyses were performed with an Agilent G1313A ALS Series 1100, with autosampler. Separation was carried out using a Waters XBridge (4.6 × 150 mm; 5 µm) column. Typical injection volume of 5 µl was used. The mobile phase consisted of (A) Acetonitrile and (B) 0.1% TFA. The flow rate of the mobile phase was maintained at 2.5 mL/min. Following standard gradient was used for a 6 minutes run:

0'0: A 0%, B 100%; 4'0 A= 50%, B=50%; 5'0: A=50%, B=50%; 5'12: A=0%, B=100%; minute 6'0: A=0%, B=100%.

Chemicals

Sodium ascorbate**,** HCl, K_2HPO_4, KH_2PO_4, DTT, acetic acid, NaCl, MePH4, ammonium sulfate, ferrous ammonium hexahydrate, MES (2-N-morpholino-ethanesulfonic acid), L-tryptophan, and catalase were purchased from Sigma-Aldrich. rhIDO1 and *N*-formyl-kynurenine were purchased from Biovision. α-Methyl-kynurenine was purchased from TRC Canada. TPH1 and TPH2 were purchased from BPS Bioscience.

General procedures

Each enzymatic reaction was accompanied by a simultaneous positive and negative control reaction. Positive controls contained the same volume and concentration of the solution of tryptophan instead of 6-F-AMTr. Negative controls contained an equal volume of buffer solution instead of the corresponding enzyme solution. All the solutions were freshly prepared a few minutes before the start of the enzymatic assay.

IDO1

The reaction mixture contained 20 mM ascorbate, 2250 units/ml catalase, 200 µM of substrate (*R*- or *S*-6-F-AMTr), 10 mM methylene blue and 100 nM of IDO1 enzyme in potassium phosphate buffer pH 6.5. The samples were incubated at 37^{o}C for 40 minutes and analyzed at HPLC.

Results

Tryptophan and 6-F-AMTr were observed at 280 nm at retention times of 3.57 min and 3.86 min respectively. The oxidation product of tryptophan, α-methyl-*N*-formyl-kynurenine, was observed at 321 nm at 3.20 min. No peak with similar retention time was observed for 6-F-AMTr even after 15h reaction. Simultaneously, nearly full conversion of tryptophan was observed after 40min, while no reduction in intensity of either enantiomer of 6-F-AMTr was observed and the HPLC chromatograms looked identical to those of the negative control samples.

TPH1 and TPH2[1]

40 μL of an 8 ng/μl enzyme solution were added to a solution of 400 mM ammonium sulfate, 2 mM DTT, 175 units/mL bovine liver catalase, 50 μM ferrous ammonium hexahydrate sulfate in 100 mM MES buffer, pH 7.0 and equilibrated for 2 minutes at 15°C. A second solution containing 120 μM of substrate (6-F-AMTr), 10 mM HCl, 12 mM DTT and 600 μM 6-MePH4 in water was also equilibrated at 15°C for 2 min. The two solutions were then mixed and incubated for 2 hours at 15°C.

Results

Tryptophan and 6-F-AMTr were observed at 280 nm at retention times 3.57 min and 3.86 min, respectively. The oxidation product of tryptophan, 5-hydroxytryptophan, was observed at 300 nm at retention at 3.07 min. No peak with similar retention time was observed and the chromatograms look identical to those of the negative control samples.

Computational studies

Molecular modeling and docking ligands

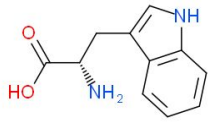
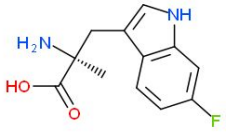
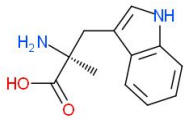
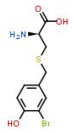
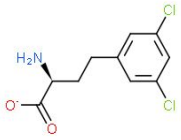
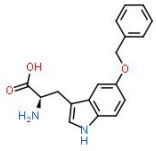
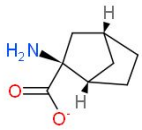
Regularized models of spatial structures of protein models were built based on the crystal structures of human LAT1 (PDB: 6IRT and 6IRS) in an inward open conformation using homology modeling tools included in the standard protocols of the ICM-Pro software package (Molsoft LLC, USA).[2] Only standard torsion angles of amino acid side chains (χ_i) were allowed to vary during energy minimization and docking of ligands. The ICM default set of energy parameters (ECEPP/3 potential) for van-der-Waals, electrostatic, torsion energy interactions, hydrogen bonding and solvation free energy were used in these calculations. Spatial structures of all LAT1 ligands (tryptophan, 6-F-AMTr, S-(3-bromo-4-methoxybenzyl)-L-cysteine, L-2-amino-4-(3,5-dichlorophenyl)-butanoic acid, 5-(benzyloxy)-tryptophan and 2-amino-2-norbornanecarboxylic acid) were generated using molecular editor of the ICM-Pro software package.

Virtual ligand screening

The docking of the ligands in target LAT1 binding pocket was performed using the ICM-Dock method, the ICMFF force field and ICM standard protocols for docking of flexible ligands as implemented in the DockScan utility of ICM-Pro software package (Molsoft LLC). The search for ligand conformations in LAT1-ligand complexes was carried out with the highest thoroughness corresponding to the number of free torsion angles of ligand (thorough = 30), which was selected on preliminary tests of the reproducibility of the docking results.[4] To improve reliability and reproducibility of the docking results 100 repeated starts of the ligand dockings were used for each ligand under investigation. The most energetically favorable poses of all ligands in LAT1 binding

site were selected for further analysis. The calculations were done using SPbPU supercomputing cluster.

Table S1. Correlation between ICM-score and IC50 values for LAT1 inhibition.

Ligand	Name	ICM-Score	IC50, uM
	Tryptophan	-19.67	20.16[2]
	6-Fluoro- α-methyl tryptophan (6-F-AMTr)	-17.74	
	α-Methyl-Tryptophan	-14.55	
	S-(3-Bromo-4-methoxybenzyl)-L-cysteine	-19.31	33.20[5]
	L-2-Amino-4-(3,5-dichlorophenyl)butanoic acid	-17.56	0.64[5]
	5-(Benzyloxy)-tryptophan	-17.59	1.48[5]
	2-Amino-2-norbornanecarboxylic acid (BHC)	-15.00	115.10[2]

Additional data on animal studies

WT mice (C57Bl/6J) were injected intravenously with [18F]AMT (n = 4) and compared with [11C]AMT scans (n = 4). 93 minute long PET measurements collected in list mode and reconstructed into 30 timeframes (4x10 s, 4x20 s, 4x60 s, 7x180 s, 11x360 s frames).

Table S2. Injected radioactivity and weight of subject animals.

	[^{18}F]AMT		**[^{11}C]AMT**		
Animal ID	Injected RA (MBq)	Weight (g)	Animal ID	Injected RA (MBq)	Weight (g)
M70-6	11.7	26.5	M30-1	4.6	28.7
M70-40	11.0	26.7	M30-3	5.4	27.8
M70-5	11.6	26.4	M30-5	9.9	30.6
M70-50	13.3	24.4	M30-9	10.2	31.5
Average	**11.9 ± 0.98**	**26 ± 1.07**	**Average**	**7.53 ± 2.94**	**29.65 ± 1.70**

References

1. Moran G.R., Fitzpatrick P.F., A continuous fluorescence assay for tryptophan hydroxylase. *Anal Biochem*, **1999,** 266(1), 148-152.
2. Yan R., Zhao X., Lei J., Lei J., Zhou Q., Structure of the human LAT1–4F2hc heteromeric amino acid transporter complex*. Nature,* **2019***,* 568**,** 127–130
3. Abagyan R., Totrov M., Biased Probability Monte Carlo Conformational Searches and Electrostatic Calculations for Peptides and Proteins. *J Mol Biol*, **1994**, 235 (3), 983-1002.
4. Kolchina N., Khavinson V., Linkova N., Yakimov A., Baitin D., Afanasyeva A., Petukhov M., Systematic search for structural motifs of peptide binding to double-stranded DNA. *Nucleic Acids Res.*, **2019**, 47 (20), 10553–10563
5. Singh, N., Scalise, M., Galluccio, M., Wieder, M., Seidel, T., Langer, T., Indiveri, C., Ecker, G.F. Discovery of Potent Inhibitors for the Large Neutral Amino Acid Transporter 1 (LAT1) by Structure-Based Methods. *Int. J. Mol. Sci.* **2019**, *20*, 27-52.