COMMUNICATION

Supporting information

Contents

1 Molecular biology methods

1.1 Plasmid construction

The target genes encoding tyrosinase from *Ralstonia solanacearum* were optimised for codon usage in *E. coli* and synthesised by DNA2.0™ (Menlo Park, CA, USA) with Pj401 vectors. The gene for tyrosinase from *Candidatus Nitrosopumilus* was synthesised from Genscript™ (NJ, USA) with a pET-29a vector. PHBH from *Pseudomonas aeruginosa*, tyrosinases from *Bacillus megaterium* and *Rhizobium meliloti*, tyrosinases and their corresponding cofactor protein form *Streptomyces avermitilis* and *Streptomyces antibioticus* were generated via PCR amplification and were ligated with a pET-29a vector. Multiple sequence alignments were constructed with Clustal omega and Sequence Manipulation Suite [1].

```
Rhizobium meliloti   ------------------------------------------------------------------------------- 0
Ralstonia solanacear MRIDFTINNGGDAAARYLTWAPSPLRLRLLDATPGPDVVATLSEDRQPNGGSIRFCATPDGNFTPTLKVPLPASGASVTV 80
Candidatus Nitrosopu ------------------------------------------------------------------------------- 0
Bacillus megaterium  ------------------------------------------------------------------------------- 0
Streptomyces avermit ------------------------------------------------------------------------------- 0
Streptomyces antibio ------------------------------------------------------------------------------- 0

Rhizobium meliloti   ----------------------------------MTSADGQKDLQ--SYMDAVTAMLKLPPS-------DRRNWY---RNG 35
Ralstonia solanacear YVRGKFGTPSQADGDVSIVVGGPASELGRLPVMVRVRKNANQLTPAERDRFISAMAQINNR-----GTGRFTDERNMHVA 155
Candidatus Nitrosopu ----------------------------------MVRKNASSLNPIERENFCKAVLTLKNTKIPGHALNRYDEEVAIHFG 46
Bacillus megaterium  ----------------------------MSNKYRVRKNVLHLTDTEKRDFVRTVLILKEK-------GIYDRYIAWHGA 44
Streptomyces avermit ----------------------------------MTVRKNQATLTADEKRRFVDALVALKRS-------GRYDEEVTTHNA 40
Streptomyces antibio ----------------------------------MTVRKNQASLTAEEKRRFVAALLELKRT-------GRYDAEVTTHNA 40

Rhizobium meliloti   F-------IHLMDCPHGDWWFTSWHRGYLGYFEETCRELSGNPDFALPYWDWTANPEVLPPLFGTILDPVNSSAYIPDHN 108
Ralstonia solanacear --------GRADQQAHGGPGFLPWHRAYLLDLERELQ--AIDPAVTLPYWRFDRPA-------PNLF--------TTDF- 209
Candidatus Nitrosopu VTSRERANLPIGDGAHGNSGFLPWHREFLCRFEHALK--SVDPTVSLPYWDWSSGDTS---DTIDIF--------NDDF- 112
Bacillus megaterium  AGKFHTPPGSDRNAAHMSSAFLPWHREYLLRFERDLQ--SINPEVTLPYWEWETDAQMQDPSQSQIW--------SADF- 113
Streptomyces avermit FIMGD--TDSGERTGHRSPSFLPWHRRFLIEFEQALQ--AVDPSVALPYWDWSTDRT----ARASLW--------APDF- 103
Streptomyces antibio FILGD--TDNGERTGHRSPSFLPWHRRFLLEFERALQ--SVDASVALPYWDWSADRS----TRSSLW--------APDF- 103

Rhizobium meliloti   RFQDIMQEPIKAYWDSLSPAQLQQQNLRGYPDFDALWSDAMASFANQPNA-RFLTAQNPKLNPATQTAVDIDTIKASLAP 187
Ralstonia solanacear --IGVPDALGTVGFSPANP--------------LQFWATD----GVQGILRRQLGA-SP----GAQAAPNILTEAQTLAL 264
Candidatus Nitrosopu --MGPAGTVNSGYFSGTGNSF----------NSNRPWIVHPSLDQTSPG-QPPLGS-TLI---RNSNILLSASTLNYLMDL 175
Bacillus megaterium  --MGGNGNPIKDFIVDTGPFA----------A--GRWTTIDEQGNPSGGLKRNFGA-TK----EAPTLPTRDDVLNALKI 174
Streptomyces avermit --LGGSGRSLDGRVM-DGPFA----------ASTGNWPVNV-RVDSRTYLRRTLGG-------GGREIPTRAEVDSVLAM 162
Streptomyces antibio --LGGTGRSRDGQVM-DGPFA----------ASAGNWPINV-RVDGRTFLRRALGA-------GVSEIPTRAEVDSVLAM 162

Rhizobium meliloti   TTFANDAGAPGLAFNSPVSSSHQVAPVGFSILEGQPHNRVH-MSVGGQSAPYGLMSQNLSPLDPIFFIHHCNIDRLWDVW 266
Ralstonia solanacear ----------------GSAYRNFR-GMQ------GNPHGSAHVSYFSGSISS-----IPTAAKDPLFFLLHCNVDRLWAKW 317
Candidatus Nitrosopu GEMARDS------LNESTYNAFR-STLEHP-----PHNHVHGVTVQGHMGW------MTSPNDPIFFLHHANVDRLWAEW 237
Bacillus megaterium  TQYDTPP------WDMTSQNSFR-NQLEGFINGPQLHNRVH-RWVGGQMGV-----VPTAPNDPVFFLHHANVDRIWAVW 241
Streptomyces avermit STYDMAP------WNSAS-DGFR-NHLEGW-RGVNLHNRVH-VWVGGQMAT------GVSPNDPVFWLHHAYIDRLWAQW 226
Streptomyces antibio ATYDMAP------WNSGS-DGFR-NHLEGW-RGVNLHNRVH-VWVGGQMAT------GVSPNDPVFWLHHAYIDKLWAEW 226

Rhizobium meliloti   TRKQQAMGLPVGPTADQQTQYDPE------PYLFYVNADGSPVSDKTRAADYLEIGDFDYDYDPGSGEEVIPVATAGRSA 340
Ralstonia solanacear QSQVGRYDANV------AAAYDAGPTPTSLLAGHNLHDTLWPWNGIVTPPRPSTAPGGAMAGSS-----CVSA------- 379
Candidatus Nitrosopu QRTHPGSSN----------YTPN---ATEPYGVHLNDPMWPWQGADTTVTTRTHTDSNASLNT-----LLPS------- 291
Bacillus megaterium  QIVHRN-QN----------YQPM---KNGPFGQNFRDPMYPWNTTPEDVMNHRKLG--YVYDI-----ELRK------- 292
Streptomyces avermit QSRHPG-SG----------YVPT---GGTPNVVDLNETMKPWNDVRPADLLDHTAH--YTFDT-----V---------- 274
Streptomyces antibio QRRHPS-SP----------YLPG---GGTPNVVDLNETMKPWNDTTPAALLDHTRH--YTFDV---------------- 273

Rhizobium meliloti   PIPALEAAVSASAAVAINKPATAKLTVSQELVDVAAKPSEQSRQFAKVSIAPPMDVGGL-------------------NF 401
Ralstonia solanacear ---------------PGNAPRVSDMLDFQGVVSSSAK---LGFAYDDVPLP---------------------------- 412
Candidatus Nitrosopu ----------------FS-----TADLVTPNDVLD-HIQ---RCGPYDTDPISKPKEFEKIPKEIIKEIIKDKEKEFGDKNP 348
Bacillus megaterium  ---------------S---------------------K---RSS------------------------------------ 297
Streptomyces avermit ------------------------------------------------------------------------------- 274
Streptomyces antibio ------------------------------------------------------------------------------- 273

Rhizobium meliloti   LVFI-----SPEGTTPDLNPDGP---DFAGSFEFFGVRHHHT-------DTVSFTIPID-----------------KALDR 450
Ralstonia solanacear ------------------------------------------------------------------------------- 412
Candidatus Nitrosopu KEIIKEIIKDKEKEFGDKNPKEIIKEIIKDKEKEFGDKNPKEIKEIIKDKEKEFGDKNPKEIKEIIKDKEKEFGDKNPKE 428
Bacillus megaterium  ------------------------------------------------------------------------------- 297
Streptomyces avermit ------------------------------------------------------------------------------- 274
Streptomyces antibio ------------------------------------------------------------------------------- 273

Rhizobium meliloti   LIDDGRLKAGEPIDFAVVVAQEGKRVEGSMPAKAQ---LTDIQVGSF-------------------------------- 494
Ralstonia solanacear ------------------------------------------------------------------------------- 412
Candidatus Nitrosopu IIETGDIKIENNKDVVEILSTPSTTVSSPKHPKEQSKETLEITNTLFDPLSKINHRLDMLENEIKGTAFIKSTERPNITK 508
Bacillus megaterium  ------------------------------------------------------------------------------- 297
Streptomyces avermit ------------------------------------------------------------------------------- 274
Streptomyces antibio ------------------------------------------------------------------------------- 273

Rhizobium meliloti   ------------------------------------ 494
Ralstonia solanacear ------------------------------------ 412
Candidatus Nitrosopu RAISKNTSTKKTTRKKTKNTKNTMPKKSNTSKRKRIS 545
Bacillus megaterium  ------------------------------------ 297
Streptomyces avermit ------------------------------------ 274
Streptomyces antibio ------------------------------------ 273
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Figure S1. DNA alignment for the selected tyrosinases from *Rhizobium meliloti, Rastonia solanacearum, Candidatus nitrosopumilus, Bacillus megaterium, Streptomyces avermitilis* and *Streptomyces antibioticu.*

The target genes encoding tyrosine decarboxylase from *Enterococcus faecalis* were optimised for codon usage in *E. coli* and synthesised by DNA2.0™ (Menlo Park, CA, USA) with Pj401 vectors. DOPA decarboxylase from *Pseudomonas putida* and tyrosine decarboxylase from *Lactobacillus brevis* were generated via PCR amplification and were ligated with a pET-29a vector. Multiple sequence alignments were constructed with Clustal omega and Sequence Manipulation Suite [1].

```
Pseudomonas putida   --------------------MTPEQFRQYGHQLIDLIADYRQTVGERPVMAQVEPGYLKAALPATAPQ--QGEP-------  52
Enterococcus faecali MKNEKLAKGEMNLNALFIGDKAENGQLYKDLLIDLVDE---HLGWR--------QNYMPQDMPVISSQERTSESYEKTVNH  70
Lactobacillus brevis MEKSNRSLKDLDLNALFIGDKAENGQLYKDLLNKLVDE---HLGWR--------KNYIPSDPNMIGPEDQNSPAFKKTVGH  70

Pseudomonas putida   FAAILDDVNNLVMPGLSHWQHP-DFYGYFPSNGTLSSVLGDFLSTGLGVLGLSWQSSPALSELEETTLDWLRQLLGLSGQ  131
Enterococcus faecali MKDVLNEISSRMRTHSVPWHTAGRYWGHMNSETLMPSLLAYNFAMLWNGNNVAYESSPATSQMEEEVGHEFAHLMSYKNG  150
Lactobacillus brevis MKTVLDQLSERIRTESVPWHSAGRYWGHMNSETLMPALLAYNYAMLWNGNNVAYESSPATSQMEEEVGQEFARLMGYDYG  150

Pseudomonas putida   WSGVIQDTASTSTLVALISARER-----------------ATDYALV--------------------------RGG--LQ  166
Enterococcus faecali WGHIVADGSLAN-LEGLWYARNIKSLPFAMKEVKPELVAGKSDWELLNMPTKEIMDLLESAEDEIDEIKAHSARSGKHLQ  229
Lactobacillus brevis WGHIVADGSLAN-LEGLWYARNIKSLPFAMKEVNPELVAGKSDWELLNMPTKEIMDLLENAGSQIDEVKKRSARSGKNLQ  229

Pseudomonas putida   AEPKPLIVYVSAHAHSSVDKAALLAGFGRDNIRLIPTDERYALRPEALQAAIEQDLAAGNQPCAVVATTGTTTTALDPL  246
Enterococcus faecali AIG---KWLVPQTKHYSWLKAADIIGIGLDQVIPVPVDHNYRMDINELEKIVRGLAEEQIPVLGVVGVVGSTEEGAVDSI  306
Lactobacillus brevis RLG---KWLVPQTKHYSWMKAADIIGIGLDQVVPVPIDSNYRMDICALESIIRKYAAEKTPILGVVGVAGSTEEGAVDGI  306

Pseudomonas putida   RPV---GEIAQANG--LWLHVDSAMAGSAMI-------------------------------LPECRWMWDGIELADSV  289
Enterococcus faecali DKIIALRDELMKDGIYYVHVDAAYGGYGRAIFLDEDNNFIPYEDLQDVHEEYGVFKEKKEHISREVYDAYKAIELAESV  386
Lactobacillus brevis DKIVALRQKLQKEGIYFYLHVDAAYGGYARALFLDEDDQFIPYKNLQKVHAENHVFTEDKEYIKPEVYAAYKAFDQAESI  386

Pseudomonas putida   VVNAHKWLGVAFDCSIYYVRDPQHLIRVMSTNPSYLQSAVDGEVKNLRDWGIPLGRRFRALKLWFMIRSEGVDALQARLR  369
Enterococcus faecali TIDPHKMGYIPYSAGGIVIQDIR-----MRDVISYFAT-----------YVFEKGADIPALLGAYILEGSKA--------  442
Lactobacillus brevis TIDPHKMGYVPYSAGGIVIQDIR-----MRDTISYFAT-----------YVFEKGADIPALLGAYILEGSKA--------  442

Pseudomonas putida   RDLDNAQWLAGQVEAAAEWEVLAPVQLQTLCIRHRPAGLEGEALDAHTKG--WAE-------------------------  422
Enterococcus faecali -----------GATAASVWAAHHVIPLNVAG----YGKLIGASIECSHHFYNFLNDLTFKVGDKEIEVHTLTHPDFNMVD  507
Lactobacillus brevis -----------GATAASVWAAHHTIPLNVTG----YGKLEGASIEGAHRYYDFLKNLKFEVAGKRISVHPLISPDFNMVD  507

Pseudomonas putida   ---------------RLNA---SGAAYVTPATLDGRWMVRVSIGALPT----------ERGDVQRLVARLQDV-------  467
Enterococcus faecali YVFKEKGNDDLVAMNKLNHDVYDYASYVKGNIYNNEFITSHTDFAIPDYGNSPLKFVNSLGFSDEEWNRAGKVTVLRAAV  587
Lactobacillus brevis YVLKEDGNDDLIEMNRLNHAFYEQASYVKGSLYGKEYIVSHTDFAIPDYGDSPLAFVESLGFSEVEWRHAGKVTIIRASV  587

Pseudomonas putida   ----------------------IKG---------------- 470
Enterococcus faecali MTPYMNDKEEFDVYAPKIQAALQEKLEQIYDVK------ 620
Lactobacillus brevis MTPYMNQRENFDYFAPRIKKAIQADLEKVYASVNQKENV 626
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Figure S2. DNA alignment for the selected tyrosine/DOPA decarboxylases from *Psuedomonas putida, Enterococcus faecalis* and *Lactobacillus brevis.*

PCR amplification

The oligonucleotide primers for target gene amplification were synthesised by Eurofins Scientific (Brussels, Belgian) and are listed in Table S1.

Table S1. Oligonucleotide primers for target gene amplification

Target protein	Gene	Oligonucleotide primers
*Pa*PHBH	*pobA*	Fw: 5'-CATAGCATATGAAGACTCAAGTCGCCATC-3' *Nde* I Rv: 5'-CATAGCTCGAGCTCGATTTCCTCGTAGGGCA-3' *Xho* I
*Sav*CoF	*melC1*	Fw: 5'-CATAGCATATGCCCGAACTCACCCGCC-3' *Nde* I Rv: 5'-CATAGCTCGAGGTTGAAGGGGACGAGCGGC-3' *Xho* I
*Sav*TYR	*melC2*	Fw: 5'-CATAGCATATGACCGTACGCAAGAACCA-3' *Nde* I Rv: 5'-CATAGGCGGCCGCGACGGTGTCGAACGTGTAGT-3' *Not* I
*San*CoF	*melC1*	Fw: 5'-CATAGCATATGCCGGAACTCACCCGT-3' *Nde* I Rv: 5'-CATAGCTCGAGGTTGGAGGGGAAGGGGAG-3' *Xho* I
*San*TYR	*melC2*	Fw: 5'-CATAGCATATGACCGTCCGCAAGAACC-3' *Nde* I Rv: 5'-CATAGCTCGAGAAGGTGTAGTGCCGGGTGT-3' *Xho* I
*Pp*DDC	*pp_2552*	Fw: 5'-CATAGCATATGACCCCCGAACAATTCC-3' *Nde* I Rv: 5'-CATAGCTCGAGGCCCTTGATCACGTCCTG-3' *Xho* I

*Lb*TyrDC	*tyrdc*	Fw: 5'-CATAGGAGCTCATGGAAAAAAGTAATCGCTCA-3' *Sac* I Rv: 5'-CATAGCTCGAGAACATTTTCCTTTTGATTAAC-3' *Xho* I
*Bm*TYR	*melA*	Fw: 5'-CATAGGAATTCATGAGTAACAAGTACAGAGTTAGAAAAA-3' *EcoR* I Rv: 5'- CATAGGCGGCCGCTGATGAACGTTTTGATTTTCTTAA -3' *Not* I
*Rm*TYR	*melC2*	Fw: 5'-CATAGGAATTCATGACCAGCGCCGATG-3' *EcoR* I Rv: 5'-CATAGGCGGCCGCGAACGAGCCCACCTGAAT-3' *Not* I

PCR amplification was performed with a Techne™ TC-512 gradient thermal cycler (NJ, USA). The DNA template for *Pa*PHBH, *Sav*CoF, *Sav*TYR, *Pp*DDC and *Lb*TyrDC was the genome of the corresponding strain. *San*CoF and *San*TYR were amplified from the generated plasmid pIJ702. The PCR reaction mixtures for each gene were prepared as follows (Table S2).

Table S2. PCR mixtures for target gene amplification

Target protein	PCR reaction mixtures
*Pa*PHBH-*pobA*, *Pp*DDC-*pp_2552*, *San*CoF-*melC1*, *San*TYR-*melC2*, *Bm*TYR-*melA*	**A)** 18 µL MilliQ water, 25 µL Q5 polymerase Mastermix (NEB), 2.5 µL forward primer, 2.5 µL reverse primer and 2 µL gDNA template for a total volume of 50 µL
*Sav*CoF- *melC1* and *Sav*TYR- *melC2*	**B)** 9 µL MilliQ water, 12.5 µL Q5 polymerase Mastermix, 1.5 µL forward primer, 1.5 µL reverse primer and 2 µL plasmid DNA template for a total volume of 26.5 µL
*Rm*TYR- *melC2*, *Lb*TyrDC-*tyrdc*	**C)** The same as **A)**, and 2.5 µL DMSO (dimethyl sulfoxide) was added to the reaction mixture due to the gDNA template (*Lactobacillus brevis*) being GC-rich.

The PCR amplification was initiated by a pre-heating at 98 °C for 30 seconds, followed by 30 cycles of denaturation, annealing and extension. The denaturation was performed at 98 °C for 10 seconds, annealing at 45-72 °C (data shown in Table S3) for 30 seconds and extension at 72 °C for 30 seconds per kb. After that, a final extension carried out at 72 °C for 10 min and the reaction was held at 4 °C. To check gene amplification, 5 µL PCR product was analysed agarose gel electrophoresis (AGE) on a 1% (w/v) agarose with 2.5 µL SYBR™ Safe DNA Gel Stain (Thermo Fisher) at a voltage of 100 V for 60 min.

Table S3. Annealing temperature for target genes

Target gene	*Pa*PHBH-*pobA*	*Sav*CoF-*melC1*	*Sav*TYR-*melC2*	*San*CoF-*melC1*	*San*TYR-*melC2*
Annealing temperature	48 °C	46 °C	57 °C	61.2 °C	61.2 °C
Target gene	*Pp*DDC-*pp_2552*	*Lb*TyrDC-*tyrdc*	*Bm*TYR-*melA*	*Rm*TYR-*melC2*	
Annealing temperature	47 °C	50.4 °C	62 °C	65 °C	

Target gene extraction

Target genes were separated from other DNA fragments by AGE. This was performed with a Bio-Rad FIGE Mapper Cell (Bio-Rad Laboratories Inc., CA, USA), equipped with a PowerPac Basic Power Supply (Bio-Rad). To identify the gene amplification, 40 µL of PCR reaction of each sample was loaded onto a 1% (w/v) agarose gel and run at a voltage of 100 V for 60 min. The gel area containing target gene was then cut off under ultraviolet (UV) light at 365 nm, and then purified using a QIAquick Gel Extraction Kit (Qiagen, Germany) according to the instructions.

Digestion of target gene and pET-29a vector

Both target genes and pET-29a vector were digested at their restriction sites as presented in table S1. To do the target gene digestion, a total volume of 50 µL mixture containing 30 µL DNA from gel extraction, 11 µL MilliQ™ water, 5 µL Cutsmart™ buffer (NEB), 2 µL endonuclease for the forward primer and 2 µL endonuclease for the reverse primer (NEB) was made and performed at 37 °C for 2 h. To digest pET-29a vector, 80 µL mixture was composed of 50 µL DNA, 10 µL MilliQ™ water, 8 µL Cutsmart™

buffer, 6 µL endonuclease for the forward primer and 6 µL endonuclease for the reverse primer and performed at 37 °C for 3 h. The digested genes and vectors were then resin purified to remove the endonucleases and buffer with QIAquick Gel Extraction Kit (Qiagen) following the instructions.

Gene ligation with digested pET-29a vector

An insert-vector ratio of 3:1 was used for cohesive end ligation (Equation 1). Vector concentration (ng/mL) and insert concentration (ng/mL) were measured by the absorbance at 260/280 nm using a NanoDrop™ 2000/2000c Spectrophotometers (Thermo Fisher).

$$\frac{vector\ mass\ (ng) \times insert\ lenghth\ (bp)}{Insert\ mass\ (ng) \times Vector\ lenghth\ (bp)} = \frac{1}{3} \qquad (Equation\ 1)$$

A total volume of 20 µL ligation mixture consisted of 50 ng pET-29a vector, 17 ng insert DNA, 2 µL 10×T4 DNA ligase buffer (NEB), 1 µL T4 DNA ligase (NEB) and MilliQ™ water to a final volume of 20 µL at 25 °C for 16 h.

Transformation of *E. coli* DH5α with constructed plasmids and generation of high-copy number plasmid

To transform the constructed plasmids into *E. coli* DH5α, a 15 µL ligation reaction was added to 50 µL *E. coli* DH5α competent cells and incubated on ice for 20 min. The mixture was then heat shocked at 42 °C for 90 s, and immediately placed back on ice for 5 min to allow the plasmids to transform into the cells. The mixture was then added to 250 µL LB media and incubated at 37 °C, 250 rpm for 1 h. Subsequently, 150 µL cell culture was plated out on LB agar plates supplemented with 50 µg/mL kanamycin. The plates were then incubated at 37 °C overnight to grow bacterial colonies, which were the successful transformants.

To confirm the presence of the target gene in the generated plasmids, a single colony of the transformants was picked and inoculated in 10 mL LB media supplemented with 50 µg/mL kanamycin for subculture at 37 °C for 16 h. The generated plasmids were then purified by a QIAprep Spin Miniprep Kit (Qiagen) following the instructions. After then, the generated plasmids were digested at the ligation sites. A total volume of 20 µL digestion mixture containing 5 µL plasmids, 9 µL MilliQ™ water, 2 µL Cutsmart™ buffer (NEB), 2 µL per endonuclease was performed at 37 °C for 2 h. The digestion reaction was analysed on 1% (w/v) agarose gel to check the DNA band for the target gene. The rest of the plasmids were stored at -20 °C. Meanwhile, 500 µL trasformants were mixed with 500 µL 50% (v/v) glycerol (Sigma-Aldrich) and stored at -80 °C.

Transformation of *E. coli* BL21 (DE3) with constructed plasmids

The transformation of *E. coli* BL21 (DE3) with the constructed plasmids followed the procedure for *E. coli* DH5α transformation as described above, but instead of 15 µL ligation reaction was added to 50 µL *E. coli* DH5α competent cells, 2 µL of constructed plasmids was added to 50 µL *E. coli* BL21 (DE3) competent cells. The transformants were selected on a LB agar plate supplemented with 50 $\mu g \cdot mL^{-1}$ kanamycin after overnight culture at 37 °C. The transformants were then inoculated in 10 mL LB medium and cultured at 37 °C. After 8 h, 500 µL transformants were mixed with 500 µL 50% (v/v) glycerol and stored at -80 °C for preservation.

1.2 Recombinant protein expression in *E. coli* BL21 (DE3)

Tyrosinase expression: Selected enzyme glycerol stocks (*E. coli* BL21 (DE3)) were plated out on agar plates supplemented with 50 µg/mL kanamycin. A single colony was then picked to inoculate into 5 mL of LB media supplemented with 50 µg/mL kanamycin and grown at 37 °C and 250 rpm overnight (8-16 h). 5 mL of the overnight cultures were inoculated into a 500 mL baffled shaking flask containing 100 mL TB media supplemented with 50 µg/mL kanamycin at 37 °C, 250 rpm until an OD_{600} of approximately 0.8. Enzyme expression was initiated by the addition of 1 mM IPTG (Sigma-Aldrich) and 100 µM $CuSO_4$ (Sigma-Aldrich) to the culture. Cells were further cultivated at 25 °C, 200 rpm for 20 h. The cells were then harvested by centrifugation at 10000 rpm for 30 min. The cell pellets were quick-frozen in liquid nitrogen, and then freeze-dried. The dry cells were stored at -20 °C.

Decarboxylase expression: Selected enzyme glycerol stocks (*E. coli* BL21 (DE3)) were plated out on agar plates supplemented with 50 µg/mL kanamycin. A single colony was then picked to inoculate into 5 mL of LB media supplemented with 50 µg/mL kanamycin and grown at 37 °C and 250 rpm overnight (8-16 h). 5 mL of the overnight cultures were then added into a 500 mL baffled shaking flask containing 100 mL TB media supplemented with 50 µg/mL kanamycin at 37 °C, 250 rpm until an OD_{600} of 0.8. Enzyme expression was initiated by the addition of 1 mM IPTG (Sigma-Aldrich) to the culture. Cells were further cultivated at 37 °C, 250 rpm for 8 h. The cells were then harvested by centrifugation at 10000 rpm for 30 min. The cell pellets were quick-frozen in liquid nitrogen, and then freeze-dried. The dry cells were stored at -20 °C.

Transaminase expression: *E. coli* BL21 (DE3) harbouring pQR801 plasmids (glycerol stock) was plated out on agar plates supplemented with 30 μg/mL kanamycin. A single colony was then picked to inoculate into 5 mL of 2xTY media supplemented with 30 μg/mL kanamycin and grown at 37 °C and 250 rpm overnight (8-16 h). 1 mL of the overnight cultures was then added into a 500 mL baffled shaking flask containing 100 mL 2xTY media supplemented with 30 μg/mL kanamycin at 37 °C, 250 rpm until an OD_{600} of 0.8. Enzyme expression was initiated by the addition of 500 μM IPTG (Sigma-Aldrich) to the culture. Cells were further cultivated at 30 °C, 250 rpm for 8 h. The cells were then harvested by centrifugation at 10000 rpm for 30 min. The cell pellets were quick-frozen in liquid nitrogen, and then freeze-dried. The dry cells were stored at -20 °C.

Wildtype Δ29*Tf*NCS expression: *E. coli* BL21 (DE3) harbouring pQR1046 plasmids (glycerol stock) was plated out on agar plates supplemented with 50 μg/mL kanamycin. A single colony was then picked to inoculate into 5 mL of LB media supplemented with 50 μg/mL kanamycin and grown at 37 °C and 250 rpm overnight (8-16 h). 4 mL of the overnight cultures were then added into a 500 mL baffled shaking flask containing 100 mL TB media supplemented with 50 μg/mL kanamycin at 37 °C, 250 rpm for 2 h, and then incubated at 25 °C, 250 rpm for 1 h. Enzyme expression was initiated by addition of 500 μM IPTG (Sigma-Aldrich) to the culture. Cells were further cultivated at 25 °C, 250 rpm for another 5 h. The cells were then harvested by centrifugation at 10000 rpm for 30 min. The cell pellets were quick-frozen in liquid nitrogen, and then freeze-dried. The dry cells were stored at -20 °C.

2 Enzyme assays

2.1 Cell lysate preparation

Cell lysates of tyrosinases and tyrosine decarboxylases preparation: Dry cells were resuspended in 50 mM HEPES buffer (pH 5.5) at a concentration of 30 g/L and disrupted by 10 cycles of sonication on ice (10 s on plus 10 s off, 12 watts output) equipped with a Process Timer. Lysed cells were centrifuged at 4 °C, 10000 rpm for 15 min. The supernatant protein concentration was measured following the standard Bradford procedure. The samples were duplicated and the average OD_{595} were used for cell lysate concentration calculations.

Cell lysates of transaminase preparation: Dry cells were resuspended in 50 mM HEPES buffer (pH 7.5) supplemented with 10 mM PLP at a concentration of 30 g/L. Then the mixture was disrupted by 10 cycles of sonication on ice (10 s on plus 10 s off, 12 watts output) equipped with a Process Timer. Lysed cells were centrifuged at 4 °C, 10000 rpm for 15 min. The supernatant protein concentration was measured following the standard Bradford procedure. The samples were duplicated and the average OD_{595} were used for cell lysate concentration calculations.

2.2 Enzyme purification

NCS purification: Dry cells (around 50 mL cell cultures) were resuspended in 20 mL of 50 mM HEPES buffer (pH 7.5) with 10% (v/v) Bugbuster™ (Merck Millipore) for 5 min. The supernatant was collected by centrifugation at 4 °C, 10000 rpm for 30 min, then filtered through a 0.2 μm cellulose acetate springe filter. A PD-10 column charged with Ni-NTA (5 mL) was washed with 10 mL of MilliQ™ water, followed by 10 mL of binding buffer (50 mM HEPES, 10 mM imidazole (Sigma-Aldrich), pH 7.5). The filtered supernatant was then passed through the Ni-NTA column, and the column was washed with wash buffer (10 mL, 50 mM HEPES, 20 mM imidazole, pH 7.5) to remove some background protein. The bound protein was then eluted with elution buffer (50 mM HEPES, 500 mM imidazole, 100 mM NaCl, pH 7.5) until all the protein was collected. The eluent containing pure enzyme was concentrated using a vivaspin (10000 MW) at 4 °C, 8000 rpm for 5 min until 2.5 mL eluent remained. Then the concentrated eluent was desalted into 3 mL of 50 mM HEPES (pH 7.5), using a Sephadex™ G-25 in PD-10 column (GE Healthcare lifesciences). To store the pure enzyme, 10% (v/v) glycerol was added. The concentration of the pure protein was measured by OD_{280} using a Nanodrop. The protein was split into different eppendorfs with 0.5 mL/each, and stored at -20 °C. To check the protein purity, the expression supernatant, flow through, wash, and eluents were examined using an SDS gel (Figure S3).

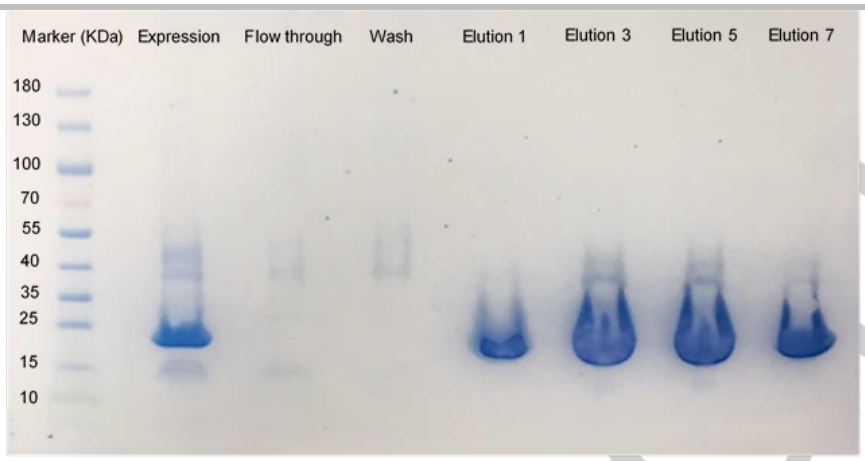


Figure S3. **SDS gel for NCS purification.** From left to right: protein marker, expression supernatant, flow through, wash and eluents.

Tyrosinases and tyrosine decarboxylases purification: Dry cells (around 100 mL cell cultures) were resuspended in 10 mL of 50 mM HEPES buffer (pH 5.5) with 10% (v/v) BugBuster™ (Merck Millipore) for 5 min, and then followed the procedure for NCS purification as described previously.

2.3 Determination of *Cn*TYR, *Ef*TyrDC and *Cv*TAm concentration in cell lysate

The recombinant proteins were expressed and analysed by SDS-PAGE (Figure S4). Then the SDS gel was analysed with a ProteinSimple™ AlphaImager™ gel documentation system, and the recombinant protein concentration present in the cell lysate was determined by AlphaView™ FluorChem Q™ software as typically: *Cn*TYR (20%), *Ef*TyrDC (22%), *Cv*TAm (50%), *Rs*TYR (20%), *Pp*PHBH (40%), *Sav*TYR (15%) *Sav*CoF (10%), *San*TYR (15%) *San*CoF (10%), *Pp*DDC (20%), *Lb*TyrDC (23%), *Rm*TYR (31%), *Bm*TYR (25%).

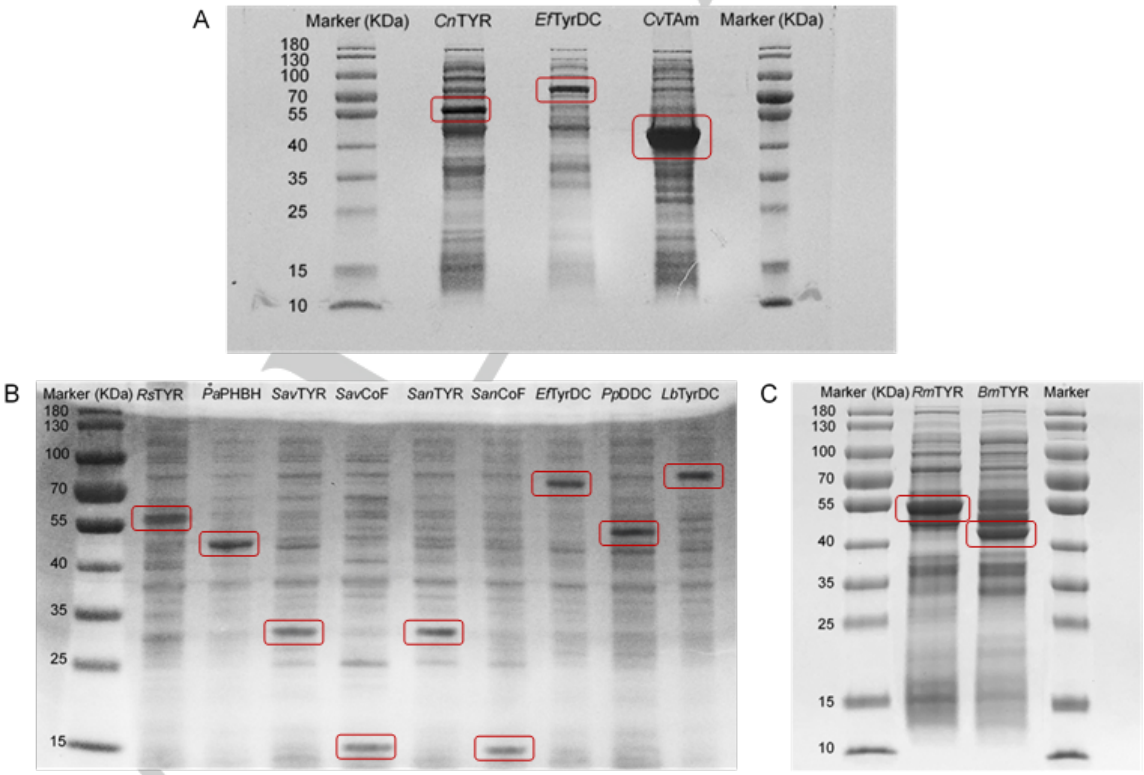


Figure S4. **SDS gel for recombinant protein expression.** From left to right: **A**. protein marker, *Cn*TYR (20%), *Ef*TyrDC (22%), *Cv*TAm (50%); **B.** *Rs*TYR (20%), *Pp*PHBH (40%), *Sav*TYR (15%) *Sav*CoF (10%), *San*TYR (15%) *San*CoF (10%), *Ef*TyrDC (22%),*Pp*DDC (20%), *Lb*TyrDC (23%); **C**. *Rm*TYR (31%), *Bm*TYR (25%)

3 General analytic methods

Chemicals: L-tyrosine **4**, tyramine **6**, L-DOPA **5**, 3-Cl-L-tyrosine **10**, 3-I-L-tyrosine **11**, sodium ascorbate **7**, sodium pyruvate and pyridoxal 5'-phosphate (PLP) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). *meta*-L-Tyrosine **8**, 3-F-L-tyrosine **9** was purchased from Alfa Aesar (Thermo Fisher Scientific Inc., MA, USA). All chemicals were purchased in the highest purity available.

Analytical HPLC: methods were performed with a Thermofisher Scientific™ Dionex™ UltiMate™ 3000 HPLC System, with a Dionex™ UltiMate™ 3000 RS Pump, a Dionex™ UltiMate™ 3000 Autosampler, a Dionex™ UltiMate™ 3000 Column Compartment and a UltiMate™ 3000 RS Diode Array Detector.

Method 1 (achiral): Achiral quantitative analyses adopted a reverse phase analysis method. Separation was achieved with an ACE 5 C18 column (150 × 4.6 mm) with a flow speed of 1 mL/min at 30 °C. The injection volume was 20 µL. Substrates and products were measured via UV absorbance at 280 nm. Eluent A (H_2O with (v/v) 0.1% TFA) and eluent B (acetonitrile) were used as a mobile phase over 10 mins and the gradient is shown below (Figure S5).

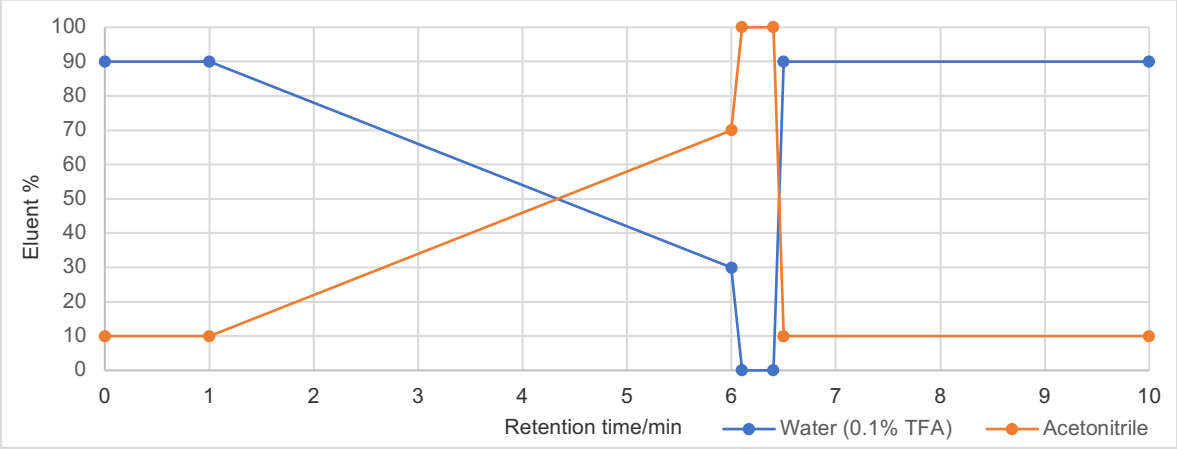


Figure S5. **Gradient of achiral analytical HPLC method**

Method 2 (chiral): The chiral separation of compound (*rac*)-**20** and (*S*)-**20**, (*rac*)-**1** and (*S*)-**1**, (*rac*)-**23** and (*S*)-**23**, (*rac*)-**25** and (*S*)-**25**, (*rac*)-**27** and (*S*)-**27**, (*rac*)-**29** and (*S*)-**29** were achieved with an Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm) or a Supelco Astec Chirobiotic™ T2 column (25 cm × 4.6 mm), and a flow speed of 1 mL/min at 30 °C. The injection volume was 5 µL. Products were measured via UV absorbance at 230 nm. Methanol (0.2% AcOH, 0.1% TEA) was used as a mobile phase over 40 min.

Method 3 (chiral): The chiral separation of compound (*rac*)-**21** and *(S)*-**21** were achieved with a Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm) and a flow speed of 0.2 mL/min at 30 °C. The injection volumes were 5 µL. Compounds were detected by UV absorbance at 230 nm. An isocratic mobile phase 20 mM NH4OAc pH 4:MeOH (70:30) was used over 120 min.

Preparative HPLC: Methods were developed with a Angilent 1260 Infinity™ HPLC System, with a 1260 Infinity™ Preparative Pump, a 1260 Infinity™ Preparative-scale Fraction Collector, a 1260 Infinity™ Multiple Wavelength Detector and a 1260 Infinity™ Preparative Autosampler.

Method 4: The separation of compounds **1**, **20**, **21**, **27** and **29** were achieved with a Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm ID x 25 cm L) preparative column or a Supelco™ Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column and a flow speed of 8 mL/min at 25 °C. The injection volume was 900 µL. Products were identified via UV absorbances at 214 nm and 280 nm. Eluent A (H_2O with 0.1% (v/v) TFA) and eluent B (acetonitrile with 0.1% (v/v) TFA) were used as a mobile phase over 28 mins and the gradient is shown below (Figure S6).

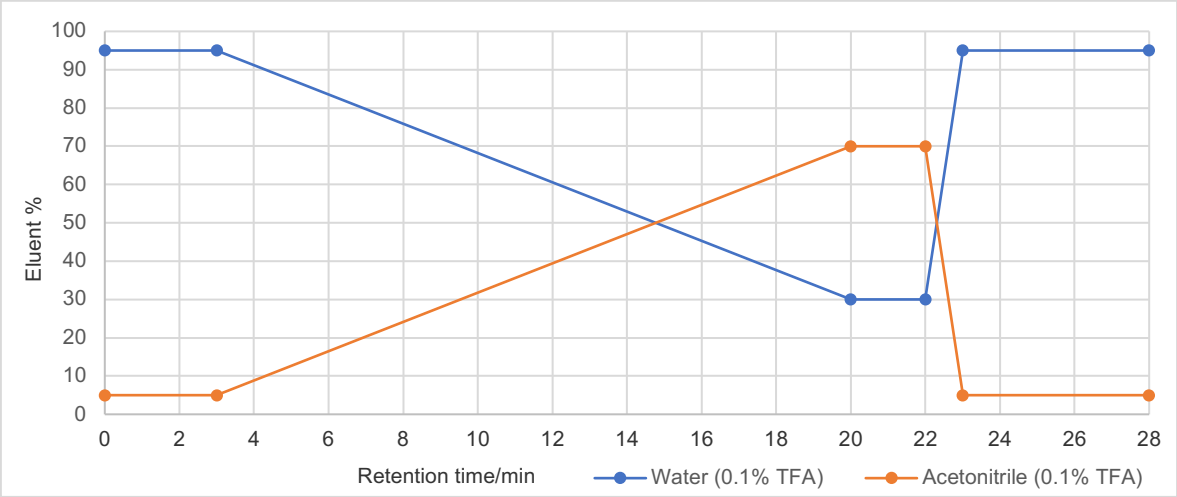


Figure S6. **Gradient of preparative HPLC method 4**

Method 5: The separation of compound **23** and **25** was achieved with a Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm ID x 25 cm L) preparative column or a Supelco™ Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column and a flow speed of 8 mL/min at 25 °C. The injection volume was 900 µL. Products were identified via UV absorbances at 214 nm and 280 nm. Eluent A (H_2O with 0.1% (v/v) TFA) and eluent B (acetonitrile with 0.1% (v/v) TFA) were used as a mobile phase over 28 mins and the gradient is shown below (Figure S7).

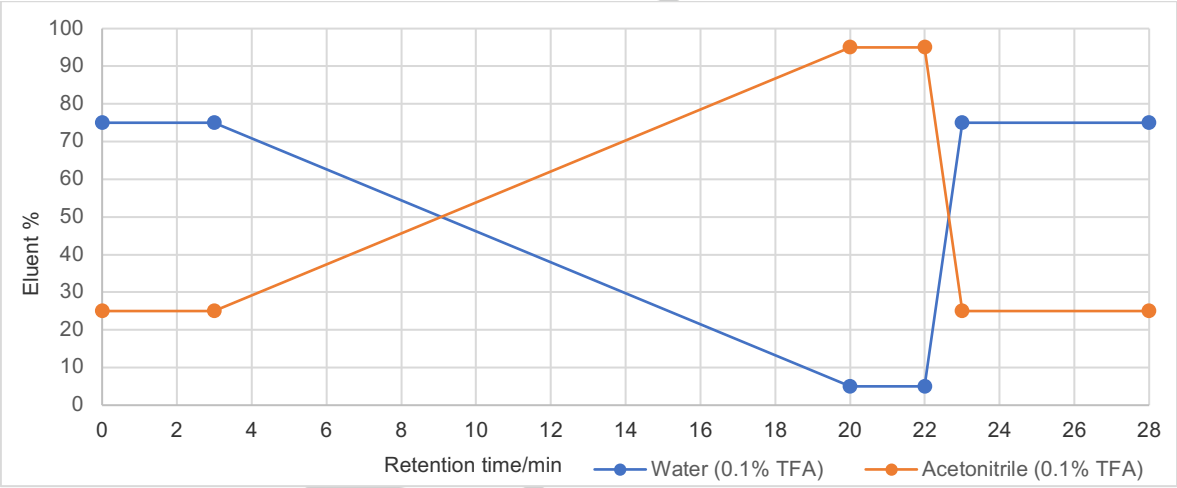


Figure S7. **Gradient of preparative HPLC method 5**

LC-MS: The molecular masses of new compounds were measured on an Agilent 1100 Series System with a Finnigan LTQ mass spectrometer. An ACE 5 C18 reverse phase column (50 mm × 2.1 mm, 5 µm) was adopted with a mobile phase of eluent A (H_2O with 0.1% (v/v) formic acid) and eluent B (acetonitrile) over 5 min with a flow rate of 0.6 mL/min. The sample injection volume was 10 µL. Chemical compounds were measured in a positive ion mode, and the operating conditions of the ESI interface were set to a capillary temperature 300 °C, capillary voltage 9 V, spray voltage 4 kV, sheath gas 40, auxillary gas 10, sweep gas 0 arbitrary units. The gradient of eluents was as follows (Figure S8).

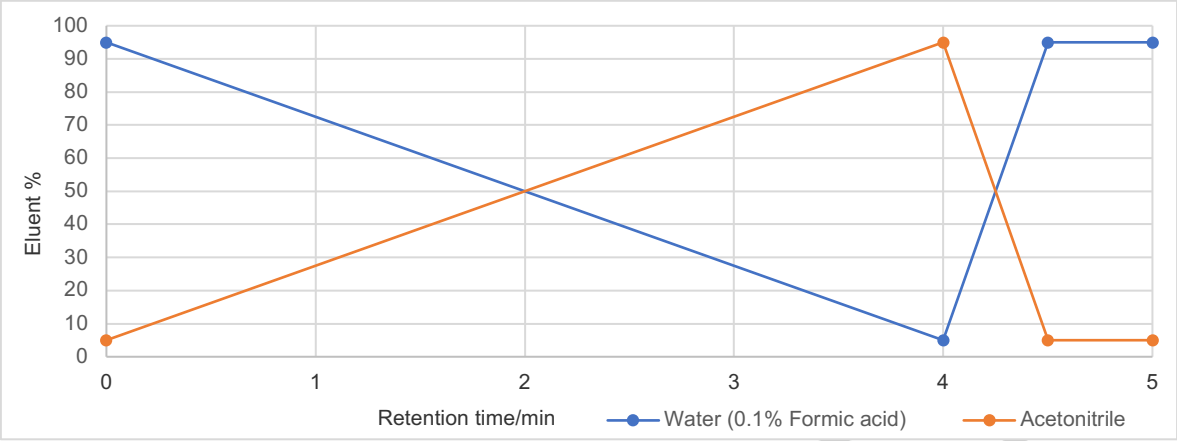


Figure S8. **Gradient of LC-MS method**

NMR spectroscopy: ^{1}H and ^{13}C NMR spectra were performed with a Bruker Avance 600 machines at 298 K. Chemical shifts (in ppm) were determined relative to tetramethylsilane (TMS) and referenced to residual protonated solvent. Coupling constants (*J*) were measured in Hertz (Hz) and multiplets of ^{1}H NMR spectroscopy couplings are shown as singlet (s), doublet (d), triplet (t) etc. Compounds **1**, **20**, **21**, **23**, **25**, **27**, **29** and **32** were characterised by NMR spectroscopy in methanol-d_4. Compound **13** was dissolved in D_2O and 20% methanol-d_4 (as a reference for the ^{13}C NMR) for NMR spectroscopy. Infrared spectra were recorded on PerkinElmer Spectrum 100 FTIR^{TM} spectrometer.

Optical rotation: The Optical rotation of products was determined by a Beillingham + Stanley^{TM} Polarimeter at the concentration stated.

4 Biocatalytic reaction

4.1 Single-step reaction

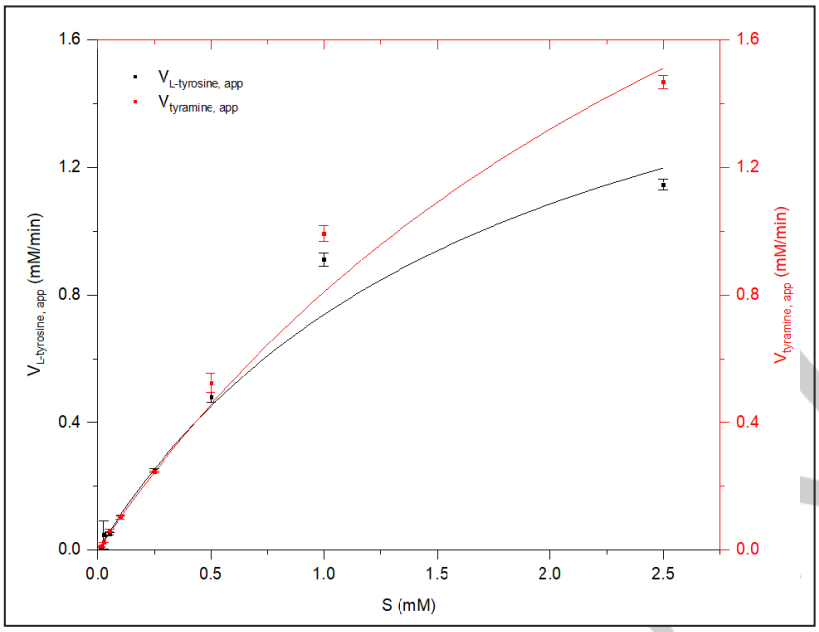
Hydroxylation reaction: The reaction mixture (450 µL, pH 5.5) for the single-step hydroxylation reaction consisted of 50 mM HEPES, 2.78 mM amino acid substrates, 11.11 mM sodium ascorbate and 1.11 mM $CuSO_4 \cdot 5H_2O$ (for TYRs), or 22.22 µM FAD and 8.33 mM NADPH (for PHBH). The reaction mixture was prepared freshly for each experiment. To initiate the enzyme reaction, 50 µL of 4 mg/mL of total protein in cell lysate (containing the % of recombinant protein indicated above in Chapter 2.3) was added to the mixture to a total volume of 500 µL, thus the amino acid substrates were at the final concentration of 2.5 mM and the cell lysate proteins were at a final concentration of 400 µg/mL respectively. In control reactions, 450 µL reaction mixture was added with 50 µL empty-vector cell lysates (pH 5.5). The experiments were performed in duplicate. Enzyme reactions were performed at 25 °C, 250 rpm for 8 h unless otherwise indicated.

Decarboxylation reaction: The reaction mixture (450 µL, pH 5.5) for the single-step decarboxylation reaction consisted of 50 mM HEPES, 2.78 mM amino acid substrates, 11.11 mM sodium ascorbate and 0.44 mM PLP. The reaction mixture was prepared freshly for each experiment. To initiate the enzyme reaction, 50 µL of 4 mg/mL of total protein in cell lysate (containing the % of recombinant protein indicated above in Chapter 2.3) was added to the mixture to a total volume of 500 µL, thus the amino acid substrates were at the final concentration of 2.5 mM and the cell lysate proteins were at the final concentration of 400 µg/mL respectively. In control reactions, 450 µL reaction mixture was added with 50 µL empty-vector cell lysates (pH 5.5). The experiments were performed in duplicates. Enzyme reactions were performed at 25 °C, 250 rpm for 8 h unless otherwise indicated.

Kinetics study

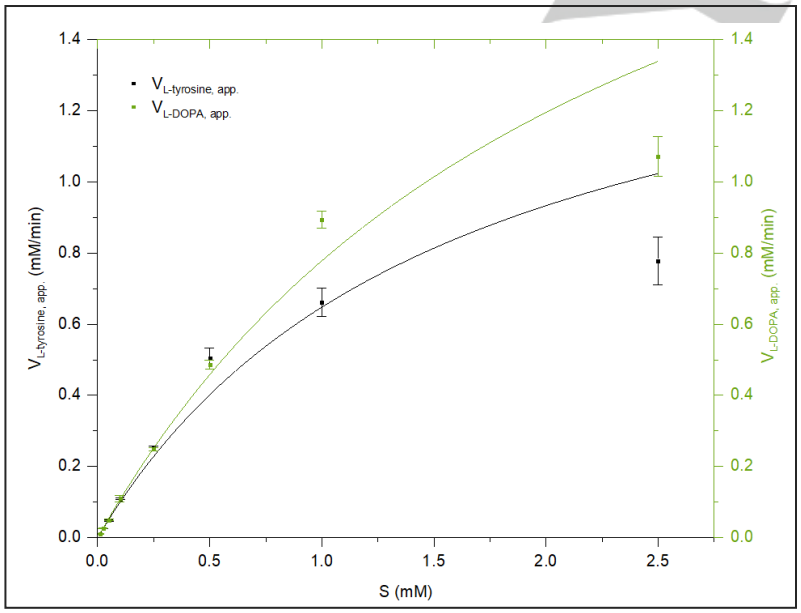
Purified *Cn*TYR and *Ef*TyrDC were used in the study at a final concentration of 50 µg/mL. Kinetics for *Cn*TYR towards L-tyrosine **4** and tyramine **6** and *Ef*TyrDC towards L-tyrosine **4** and L-DOPA **5** were measured. Due to the limitation of the substrate solubility (**4**: 2.5 mM in H_2O), we calculated the apparent K_m and k_{cat} using an Origin Software (with fitting with the Michaelis-Menten Function). Substrates were catalysed at a concentration gradient (S) of 0.01 mM, 0.025 mM, 0.05 mM 0.1 mM, 0.25 mM, 0.5 mM, 1 mM, 2 mM, 2.5 mM, and were sampled at 0 min, 1 min, 2 min, 3 min, 4 min, 5 min, 10 min, 15 min, 20 min and 30 min. Each reaction was performed in triplicate and stopped with 0.1% (v/v) TFA water. The product concentration of each sample was calculated by HPLC. A serial of curve of product concentration changing with time in different reactions was constructed, and the maximum gradient of the product concentration/time curve was calculated, which was the initially reaction velocity (V_0). From the Michealis-Menten curve (Equation 2, Figure S8 and S9), the apparent K_m and $k_{cat.}$ were calculated by Origin Software.

$$V = \frac{Vmax \cdot [s]}{Km + [s]} = \frac{Kcat \cdot [E]_t[s]}{Km + [s]} \qquad (Equation\ 2)$$



Model	Michealis-Menten Fit	
Plot	$V_{L\text{-tyrosine, app.}}$	$V_{tyramine, app.}$
$V_{max, app.}$ (mM/min)	2.05 ± 0.29	3.58 ± 0.51
$K_{m, app.}$ (mM)	1.78 ± 0.39	3.43 ± 0.58
$K_{cat, app.}$ (S^{-1})	31.6 ± 6.9	55.2 ± 9.3
R^2 (COD)	0.99	0.99

Figure 9. **Fitting of the Michaelis-Menten Function for *Cn*TYR using Origin Software.** The apparent K_m and the k_{cat} were calculated by Origin Softtware. The curve in black represents the Michaelis-Menten fit towards L-tyrosine **4.** The curve in red represents the Michaelis-Menten fit towards tyramine **6**.

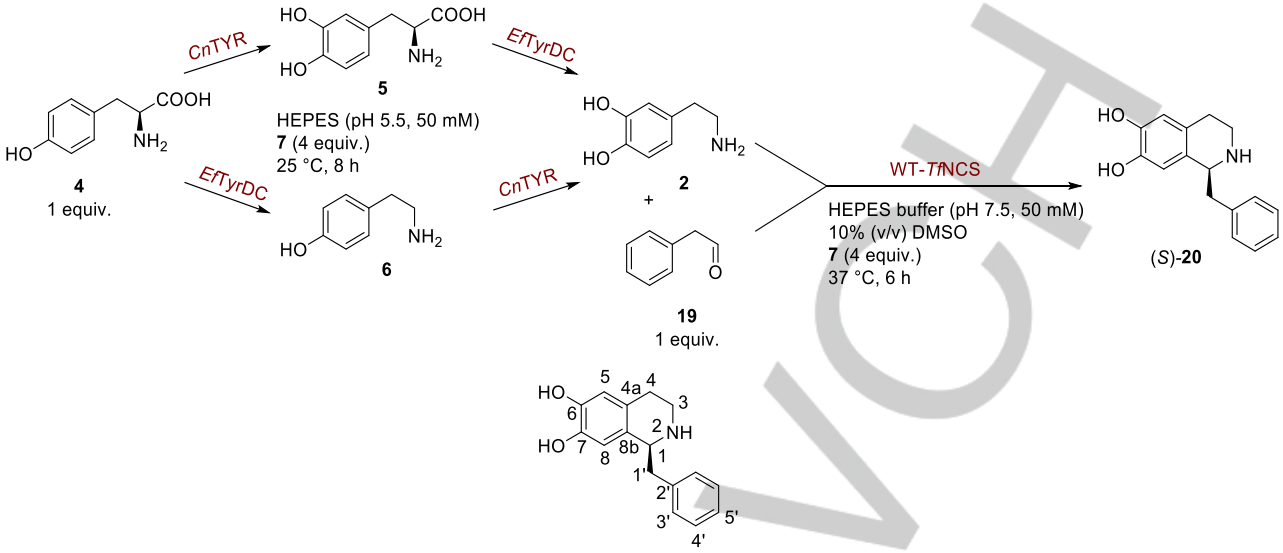


Model	Michealis-Menten Fit	
Plot	$V_{L\text{-tyrosine, app.}}$	$V_{L\text{-DOPA, app.}}$
$V_{max, app.}$ (mM/min)	1.67 ± 0.52	2.58 ± 0.59
$K_{m, app.}$ (mM)	1.58 ± 0.55	2.31 ± 0.61
$K_{cat, app.}$ (S^{-1})	39.0 ± 13.6	60.2 ± 15.9
R^2 (COD)	0.98	0.99

Figure 10. **Fitting of the Michaelis-Menten Function for *Ef*TYR using Origin Software.** The apparent K_m and the $K_{cat,}$ were calculated by Origin Software. The curve in black represents the Michaelis-Menten fit towards L-tyrosine **4.** The curve in green represents the Michaelis-Menten fit towards dopamine **2**.

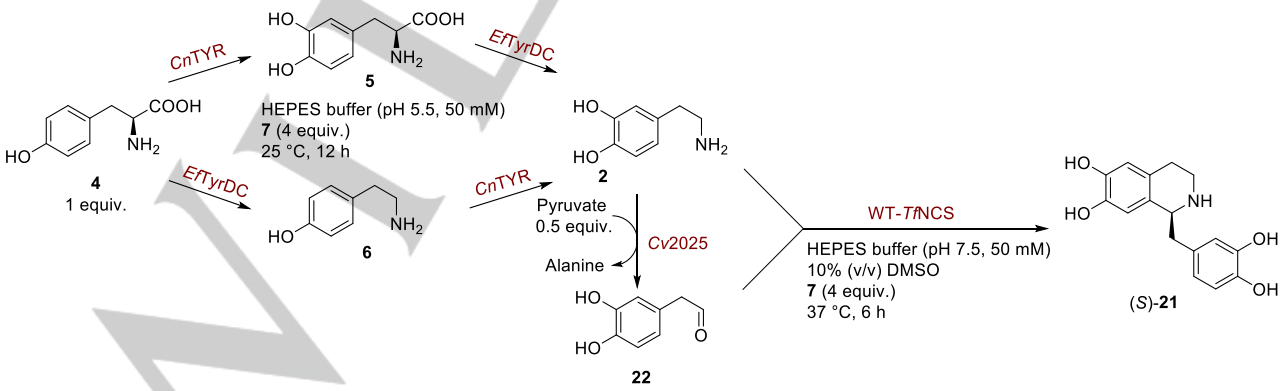
4.2 Multi-step cascade reaction scale-up

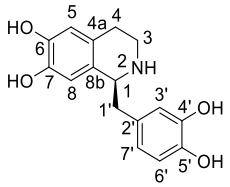
Entry 1: (*S*)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*S*)-**20**[3]



The reaction mixture (50 mL, pH 5.5) consisted of 10% (v/v) DMSO, 50 mM HEPES, 2.5 mM L-tyrosine **4**, 10 mM sodium ascorbate, 100 µM $CuSO_4 \cdot 5H_2O$ and 1.25 mM PLP. To initiate the hydroxylation and decarboxylation steps, 10% (v/v) of *Cn*TYR cell lysate and 10% (v/v) of *Ef*TyrDC cell lysate were added to the reaction mixture which was incubated at 25 °C, 250 rpm for 8 h. Then the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and 2.5 mM phenylacetaldehyde **19** was added. The Pictet-Spengler condensation was performed with 50 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 6 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 4, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 16.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**20** as a yellow powder (yield by HPLC (calibration curve) 99% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.3 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 21 mg, 66%; *e.e.* > 97%, chiral HPLC, method 2, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 12 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 7.41-7.32 (5H, m, Ph), 6.63 (1H, s, 5-H), 6.59 (1H, s, 8-H), 4.67 (1H, dd, *J* = 8.9 Hz, 5.7 Hz, 1-H), 3.49-3.44 (2H, m, 3-HH and 1'-HH), 3.29-3.24 (1H, m, 3-HH), 3.08? (1H, dd, *J* = 14.4.0 Hz, 8.9 Hz, 1'-HH), 3.02-2.98 (1H, m, 4-HH), 2.94-2.89 (1H, m, 4-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 146.9, 145.8, 136.7, 130.6, 130.2, 128.8, 123.7, 123.6, 116.2, 114.2, 57.8, 41.3, 40.9, 25.7; *m/z* [ES+] 256 ([M+H]$^+$, 100%); $[\alpha]_D^{25}$ -20.4 (c 0.50, MeOH).

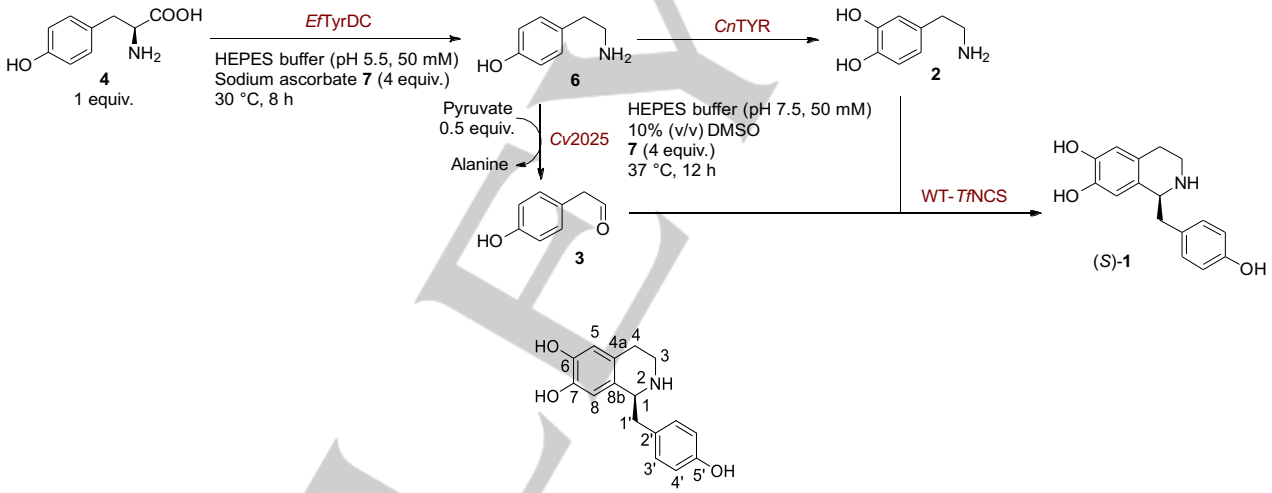
Entry 2: (*S*)-Norlaudanosoline (*S*)-**21**[2]





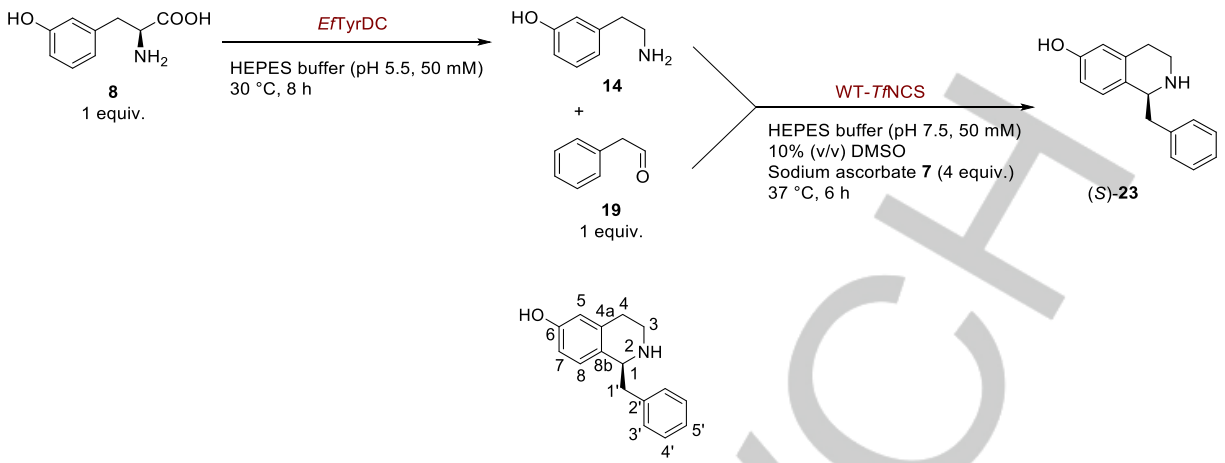
The reaction mixture (50 mL, pH 5.5) consisted of 50 mM HEPES, 2.5 mM L-tyrosine **4**, 10 mM sodium ascorbate **7**, 100 µM $CuSO_4 \cdot 5H_2O$, 1.25 mM PLP and 1.25 mM sodium pyruvate. To initiate the hydroxylation and decarboxylation steps, 10% (v/v) of *Cn*TYR cell lysate and 10% (v/v) of *Ef*TyrDC cell lysate were added to the reaction mixture which was incubated at 25 °C, 250 rpm for 8 h. Then the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and the next steps performed with 10% (v/v) of *Cv*TAm lysate and 50 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 6 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 4, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 15.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**21** as a grey powder (yield by HPLC (calibration curve) 98% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.4 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 19 mg, 53%; *e.e.* >97%, chiral HPLC, method 3, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 80 min, run time: 100 mins, flow rate: 0.2 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 6.78 (1H, d, *J* = 7.8 Hz, 6'-H), 6.74 (1H, d, *J* = 1.8 Hz, 3'-H), 6.64-6.62 (3H, m, 5-H, 8-H and 7'-H), 4.54 (1H, dd, *J* = 9.0 Hz, 5.4 Hz, 1-H), 3.44 (1H, app. quintet, *J* = 6.3 Hz, 3-HH), 3.35-3.32 (1H, m, 1'-HH), 3.25-3.21 (1H, m, 3-HH), 3.01-2.86 (3H, m, 4-HH, 4-HH and 1'-HH); ^{13}C (151 MHz; CD_3OD) δ = 147.1, 146.9, 146.1, 145.8, 127.6, 123.8, 123.6, 121.8, 117.4, 116.9, 116.1, 114.1, 58.0, 41.0, 40.7, 25.7; *m/z* [ES+] 288 ([M+H]$^+$, 100%); $[\alpha]_D^{23}$ -34.9 (c 2.5, MeOH), Lit. $[\alpha]_D$ -21 ($CHCl_3$)[4].

Entry 3: (*S*)-Norcoclaurine (*S*)-**1**[3]



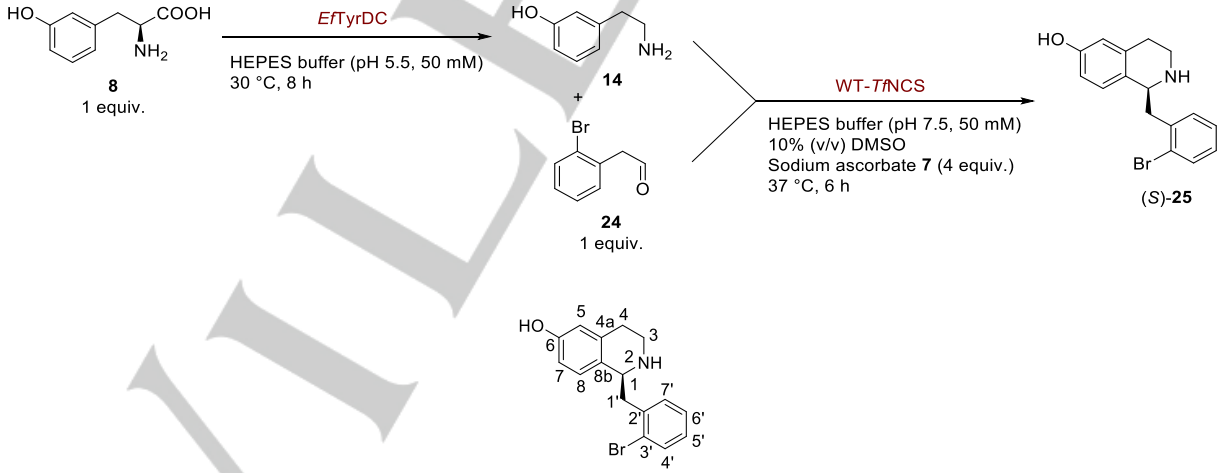
The reaction mixture (50 mL, pH 5.5) consisted of 50 mM HEPES, 2.5 mM L-tyrosine **4**, 10 mM sodium ascorbate, 100 µM $CuSO_4 \cdot 5H_2O$, 1.25 mM PLP **31** and 1.25 mM sodium pyruvate **32**. To initiate the decarboxylation step, 10% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture which was incubated at 25 °C, 250 rpm for 8 h. Then, the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and the next steps performed with 10% (v/v) of *Cn*TYR cell lysate, 10% (v/v) of *Cv*TAm lysate and 50 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 12 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 4, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 15.5 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**1** as a white powder (yield by HPLC (calibration curve) 85% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.8 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 21 mg, 62%; *e.e.* > 97%, chiral HPLC, method 2, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 11.6 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 7.13 (2H, d, *J* = 8.4 Hz, 3'-H and 7'-H), 6.80 (2H, d, *J* = 8.4 Hz, 4'-H and 6'-H), 6.62 (1H, s, 5-H), 6.61 (1H, s, 8-H), 4.57 (1H, dd, *J* = 9.3 Hz, 5.7 Hz, 1-H), 3.45 (1H, app. quintet, *J* = 6.2 Hz, 3-HH), 3.36 (1H, dd, *J* = 14.7 Hz, 5.7 Hz, 1'-HH), 3.26-3.22 (1H, m, 3-HH), 3.00-2.87 (3H, m, 4-HH, 4-HH and 1'-HH); ^{13}C (151 MHz; CD_3OD) δ = 158.2, 146.8, 145.8, 131.7, 127.0, 123.7, 123.6, 117.0, 116.2, 114.2, 57.9, 40.9, 40.5, 25.7; *m/z* [ES+] 272 ([M+H]$^+$, 100%); $[\alpha]_D^{23}$ -21.2 (c 1.5, MeOH), lit. $[\alpha]_D^{25}$ -24.0 (c 1.0, MeOH).[5]

Entry 4: (*S*)-1-Benzy-1,2,3,4-tetrahydroisoquinoline-6-ol (*S*)-**23**[3]



The reaction mixture (40 mL, pH 5.5) consisted of 10% (v/v) DMSO, 50 mM HEPES, 10 mM *meta*-L-tyrosine **8**, 40 mM sodium ascorbate, and 5 mM PLP. To initiate the decarboxylation step, 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction which was incubated at 25 °C, 250 rpm for 8 h. Then, the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and the Pictet-Spengler condensation performed with 10 mM phenylacetaldehyde **19** and 100 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 16 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 5, Vydac^{TM} 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 12.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**23** as a white powder (yield by HPLC (calibration curve) 82% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.9 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 24 mg, 25%; *e.e.* >97%, chiral HPLC, method 2, Supelco Astec Chirobiotic^{TM} T2 column (25 cm × 4.6 mm), retention time: 18.0 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 7.40-7.30 (5H, m, Ph-H), 6.97-6.96 (1H, m, Ar-H), 6.67-6.65 (2H, m, Ar-H), 4.72 (1H, dd, *J* = 8.4 Hz, 6.6 Hz, 1-H), 3.53-3.46 (2H, m, 3-HH and 1'-HH), 3.34-3.32 (1H, m, 3-HH), 3.12-3.07 (2H, m, 1'-HH and 4-HH), 3.03-2.98 (1H, m, 4-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 158.6, 136.7, 134.0, 130.7, 130.2, 129.2, 128.8, 123.5, 116.1, 115.6, 57.8, 41.2 40.6 26.4; *m/z* [ES+] 240 ([M+H]$^+$, 100%); $[\alpha]_D^{25}$ -12.6 (0.35, MeOH).

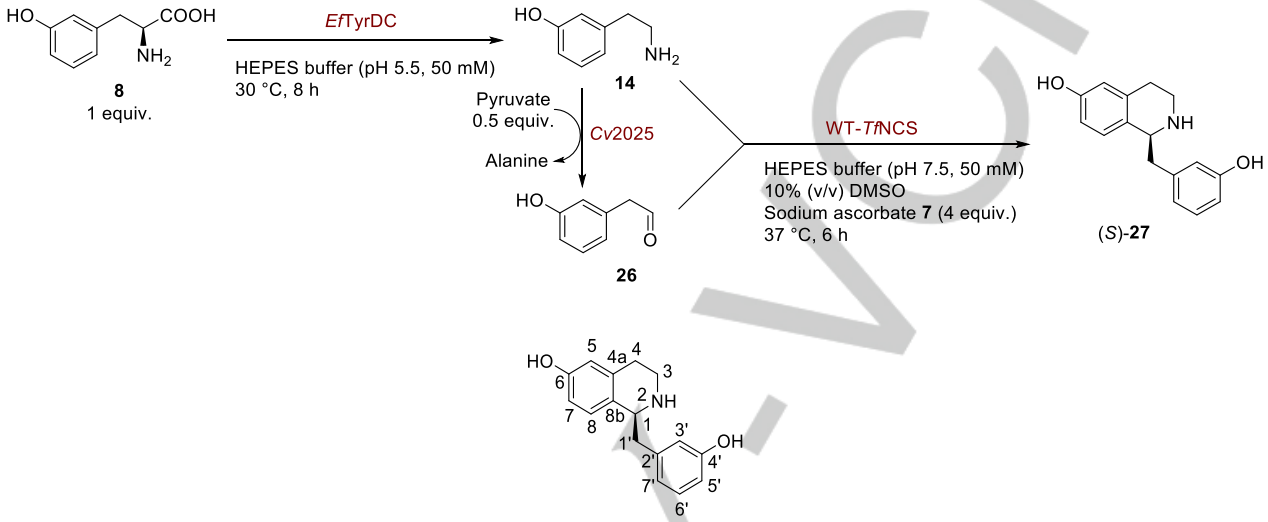
Entry 5: (*S*)-1-(2-Bromobenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**25**



The reaction mixture (40 mL, pH 5.5) consisted of 10% (v/v) DMSO, 50 mM HEPES, 10 mM *meta*-L-tyrosine **8**, 40 mM sodium ascorbate, and 5 mM PLP. To initiate the decarboxylation step, 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture and incubated at 25 °C, 250 rpm for 8 h. Then, the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and the Pictet-Spengler condensation was performed with 10 mM 2-bromo-phenylacetaldehyde **24** and 100 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 16 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 5, Supelco^{TM} Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column, retention time: 14.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**25** (yield by HPLC (calibration curve) 45% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 6.0 min, run time: 10 min, flow rate: 1 mL/min); final isolated yield 36 mg, 28%; *e.e.* 75%, chiral HPLC, method 2, Supeclo Astec Chirobiotic^{TM} T2 column

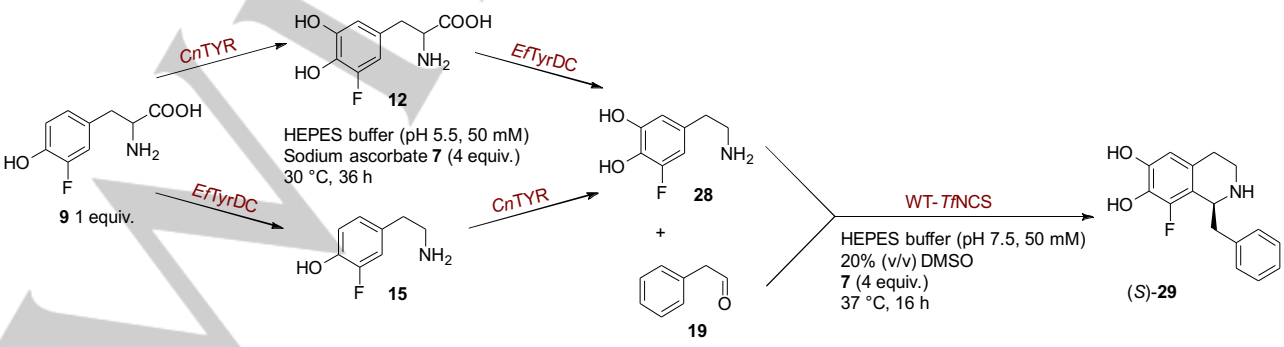
(25 cm × 4.6 mm), retention time: 18 min and 22 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 7.65 (1H, dd, *J* = 8.4 Hz, 1.2 Hz, 4'-H), 7.35-7.23 (3H, m, Ar-H), 6.80 (1H, d, *J* = 8.4 Hz, 7-H), 6.67 (1H, d, *J* = 2.4 Hz, 5-H), 6.62 (1H, dd, *J* = 8.4, 2.4 Hz, 8-H), 4.80 (1H, dd, *J* = 8.4 Hz, 6.6 Hz, 1-H), 3.64-3.57 (2H, m, 3-HH and 1'-HH), 3.37-3.33 (1H, m, 3-HH), 3.26 (1H, dd, *J* = 14.4 Hz, 8.4 Hz, 1'-HH), 3.17-3.12 (1H, m, 4-HH), 3.05-3.00 (1H, m, 4-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 158.7, 136.0, 134.5, 134.1, 133.6, 130.8, 129.3, 129.2, 126.1, 122.9, 116.2, 115.6, 55.9, 41.6, 40.4 26.3; *m/z* [ES+] 318 ($[M+H]^+$, 100%); *m/z* [HRMS ES+] found $[M+H]^+$ 318.0502. $[C_{16}H_{16}{}^{79}BrNO+H]^+$ requires 318.0493; $[\alpha]_D^{25}$ -11 (0.20, MeOH).

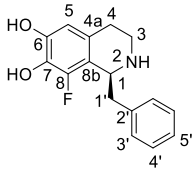
Entry 6: (*S*)-1-(3-Hydroxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**27**[2]



The reaction mixture (40 mL, pH 5.5) consisted of 50 mM HEPES, 10 mM *meta*-L-tyrosine **8**, 40 mM sodium ascorbate, 5 mM PLP and 5 mM sodium pyruvate. To initiate the decarboxylation step, 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture which was incubated at 25 °C, 250 rpm for 8 h. Then, the reaction was adjusted to pH 7.5 with 2.5 M NaOH, and the and the next steps performed with 20% (v/v) of *Cv*TAm and 100 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 16 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 4 VydacTM 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 17.1 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**27** (yield by HPLC (calibration curve) 78% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.2 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 32 mg, 32%; *e.e.* 95% chiral HPLC, method 2, Supelco Astec Chirobiotic^{TM} T column (25 cm × 4.6 mm), retention time: 14 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD3OD) δ = 7.20 (1H, t, *J* = 7.8 Hz, 6'-H), 7.02 (1H, d, *J* = 9.0 Hz, 8-H), 6.79-6.75 (3H, m, 3'-H, 5'-H and 7'-H), 6.70-6.65 (2H, m, 5-H and 7-H), 4.68 (1H, dd, *J* = 8.4 Hz, 6.0 Hz, 1-H), 3.52-3.47 (1H, m, 3-HH), 3.41 (1H, dd, *J* = 14.4 Hz, 6.0 Hz, 1'-HH), 3.30-3.28 (1H, m, 3-HH), 3.11-3.06 (1H, m, 1'-HH), 3.03-2.97 (2H, m, 4-HH and 4-HH); ^{13}C NMR (151 MHz; CD3OD) δ = 159.3, 158.6, 138.0, 134.0, 131.3, 129.1, 123.5, 121.5, 117.4, 116.0, 115.7, 115.6, 57.8, 41.2, 40.6, 26.4; m/z [ES+] 256 ([M+H]+, 100%); $[\alpha]_D^{25}$ 5.8 (0.45, MeOH).

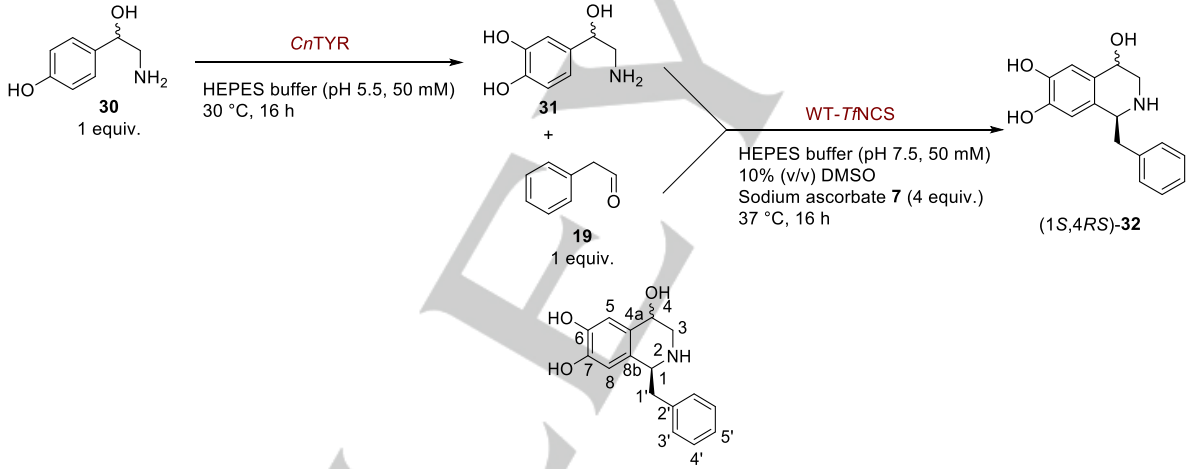
Entry 7: (*S*)-1-Benzyl-8-fluoro-1,2,3,4-tetrahydroisoquinoline-6,7-diol (*S*)-**29**





The reaction mixture (40 mL, pH 5.5) consisted of 20% (v/v) DMSO, 50 mM HEPES, 15 mM 3-F-L-tyrosine **9**, 60 mM sodium ascorbate **7**, 100 µM $CuSO_4 \cdot 5H_2O$ and 7.5 mM PLP. To initiate the hydroxylation and decarboxylation steps, 20% (v/v) of *Cn*TYR cell lysate and 20% (v/v) of *Ef*TyrDC cell lysate were added to the reaction mixture which was incubated at 25 °C, 250 rpm for 24 h. Then, the reaction was adjusted to pH 7.5 with 2.5 M NaOH and 20% (v/v) DMSO and 5 mM phenylacetaldehyde **19** were added to the reaction mixture. The Pictet-Spengler condensation was performed with 100 µg/mL of *Tf*NCS at 37 °C, 250 rpm for 16 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was purified using preparative HPLC (method 4, Supelco™ Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column, retention time: 18.6 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**29** (yield by HPLC (calibration curve) 35% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.5 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 38 mg, 23%; *e.e.* 90%, chiral HPLC, method 2, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 12 min, run time: 40 mins, flow rate: 1 mL/min). ^{1}H NMR (600 MHz; CD_3OD) δ = 7.39-7.30 (5H, m, Ph-H), 6.51 (1H, s, 5-H), 4.91 (1H, dd, *J* = 9.5 Hz, 4.3 Hz, 1-H), 3.50 (1H, ddd, *J* = 12.6 Hz, 8.4 Hz, 6.6 Hz, 3-HH), 3.41 (1H, dd, *J* = 15.0 Hz, 4.3 Hz, 1'-HH), 3.29-3.26 (1H, m, 3-HH), 3.16 (1H, dd, *J* = 15.0 Hz, 9.5 Hz, 1'-HH), 2.97-2.93 (2H, m, 4-HH and 4-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 150.2 (d, $^1J_{CF}$ = 238.6 Hz), 149.1 (d, $^3J_{CF}$ = 7.6 Hz), 136.5, 133.8 (d, $^2J_{CF}$ = 15.1 Hz), 130.5, 130.3, 128.9, 123.2 (d, $^3J_{CF}$ = 4.5 Hz), 112.1 (d, $^2J_{CF}$ = 12.1 Hz), 111.8 (d, $^4J_{CF}$ = 1.5 Hz), 53.3, 39.9, 38.9, 25.2; *m/z* [ES+] 274 ($[M+H]^+$, 100%); *m/z* [HRMS ES+] found $[M+H]^+$ 274.1234. $[C_{16}H_{16}FNO_2+H]^+$ requires 274.1243; $[\alpha]_D^{25}$ 21 (c 1.1, MeOH).

Entry 8: (1*S*,4*RS*)-1-Benzyl-1,2,3,4-tetrahydroisoquinoline-4,6,7-triol (1*S*,4*RS*)-**32**



The reaction mixture (20 mL, pH 5.5) consisted of HEPES (50 mM), (*rac*)-octopamine **30** (40 mM), sodium ascorbate (80 mM), and $CuSO_4$ (100 µM) To initiate the hydroxylation step, 20% (v/v) of *Cn*TYR cell lysate was added to the reaction which was incubated at 25 °C, 250 rpm for 16 h. Then, the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH and 10% (v/v) DMSO was added. The Pictet-Spengler condensation was performed with phenylacetaldehyde **19** (40 mM) and *Tf*NCS (100 µg/mL) at 37 °C, 250 rpm for 16 h. In control reactions, all the enzymes were replaced by empty-vector cell lysate. The product was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a yellow oil. The residue was resuspended in 10 mL of a 1:1 mixture of H_2O:acetonitrile and the solution freeze-dried to (1*S*,4*RS*)-**32** (65% yield by HPLC (calibration curve) (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.9 min (1*S*,4*R*)-**32** and 5.1 min (1*S*,4*S*)-**32**, run time: 10 mins, flow rate: 1 mL/min; ratio (1*S*,4*R*): (1*S*,4*S*) = 5:3; final isolated yield 102 mg, 47%. Major product (1*S*,4*R*): ^{1}H NMR (600 MHz; CD_3OD) δ = 7.33-7.31 (5H, m, Ph-H), 6.84 (1H, s, 5-H), 6.67 (1H, s, 8-H), 4.57 (1H, app. t, *J* = 3.9 Hz, 4-H), 4.27 (1H, dd, *J* = 8.4 Hz, 4.8 Hz, 1-H), 3.21-3.16 (2H, m, 3-HH and 3-HH), 3.07-3.02 (2H, m, 1'-HH and 1'-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 146.7, 146.0, 138.7, 130.6, 129.9, 128.6, 128.3, 128.0, 116.7, 113.6, 65.5, 58.0, 49.1, 42.2; Minor product (1*S*,4*S*): ^{1}H NMR (600 MHz; CD_3OD) δ = 7.29-7.24 (5H, m, Ph-H), 6.89 (1H, s, 5-H), 6.53 (1H, s, 8-H), 4.60 (1H, app. t, *J* = 5.1 Hz, 4-H), 4.32 (1H, dd, *J* = 7.5 Hz, 5.7 Hz, 1-H), 3.37-3.33 (2H, m, 3-HH and 3-HH), 2.99-2.90 (2H, m, 1'-HH and 1'-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 146.5, 146.1, 138.6, 129.4, 129.2, 128.7, 128.3, 128.1, 116.2, 113.8, 65.2, 57.6, 48.0, 42.0; *m/z* [ES+] 272 ($[M+H]^+$, 100%); *m/z* [HRMS ES+] found $[M+H]^+$ 272.1279. $[C_{16}H_{17}NO_3+H]^+$ requires 272.1287

To investigate whether the product ratio was selected by the NCS enzyme, wild-type *Tf*NCS and seven selected mutants (100

μg/mL) were used in cascade reaction (total 500 μL reaction volume with 10 mM **30,** 40 mM **7**, 100 μM $CuSO_4$ and 10 mM **19**, procedures were the same as that described above). The results indicated that all the variants used gave the product **32** in a similar ratio of (1*S*,4*R*): (1*S*,4*S*) = 5:3, with WT, A79F and A79I giving the highest yields.

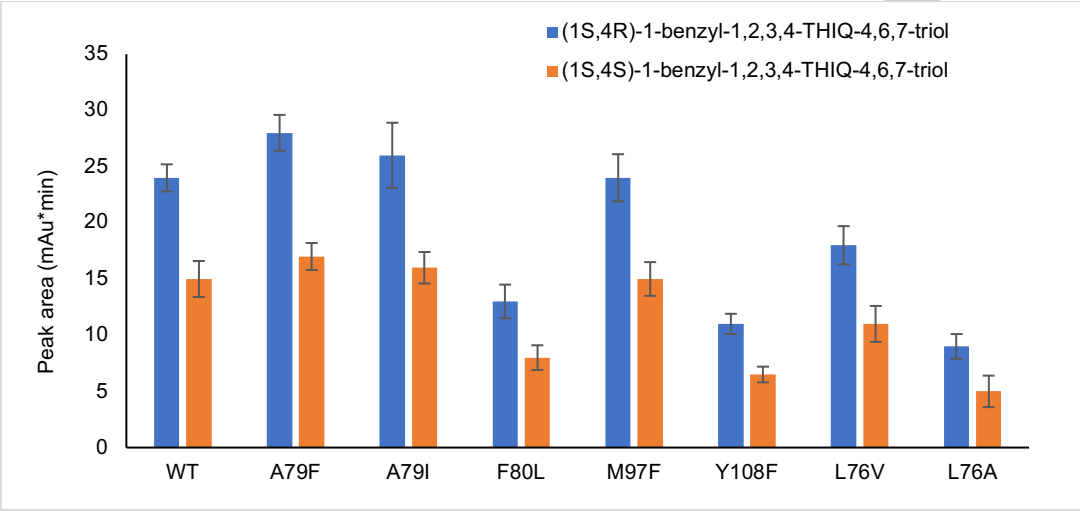
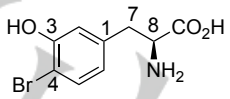


Figure S11. **Wild-type *Tf*NCS and seven NCS mutants screening to form 1-benzyl-1,2,3,4-tetrahydroisoquinoline-4,6,7-triol 32**

All the samples after the reaction were stored at -20 °C, until analysis by HPLC or LC-MS. Note that the absolute stereochemistries of the 2 isomers of **32** were confirmed by using (*R*)-norepinaphrine in a reaction with **19** and *Tf*NCS (85% yield by HPLC (calibration curve)).

2-Amino-3-(4-bromo-3-hydroxyphenyl) propanoic acid **13**[6]



L-*meta*-tyrosine **8** (100 mg, 0.55 mmol) was suspended in AcOH (1 mL) and HBr (48% in H_2O, 1.2 mL, 10.8 mmol) was added. After 5 minutes, DMSO (48 uL, 0.66 mmol) was added to the reaction mixture. The suspension was warmed to 65 ºC for 2 h, then stirred at room temperature for 18 h. The solvent was evaporated *in vacuo* to obtain a solid which was dissolved in the minimum amount of hot water, the pH was adjusted to 6 (sat. $NaHCO_3$) and then cooled to 0 ºC. The suspension was filtered, washed (cold water) and dried to give **13** (94 mg, 65%); ^{1}H NMR (600 MHz; CD_3OD) δ = 7.40 (d, *J* = 8.7 Hz, 1H, 5-H), 6.86 (s, 1H, 2-H), 6.72 (d, *J* = 8.7 Hz, 1H, 6-H), 4.26 (t, *J* = 7.5 Hz, 1H, 8-H), 3.40 (dd, *J* = 14.1, 7.5 Hz, 1H, 7-HH), 3.16 (dd, *J* = 14.1, 7.5 Hz, 1H, 7-HH); ^{13}C NMR (151 MHz; CD_3OD) δ = 171.5, 158.2, 136.2, 135.1, 120.0, 118.2, 114.6, 53.9, 38.1; *m/z* [ES+] 259 ([M+H]$^+$, 100%); *m/z* [ES+] 259 ([M+H]$^+$, 100%); $[\alpha]_D^{24}$ 8.5 (c 1.4, 1 M HCl), lit $[\alpha]_D^{23}$ 2.6 (c 5.0, 1 M HCl)[6].

4.3 Larger scale-up reactions for Entries 2 and 6

Entry 2: The reaction mixture (2 L, pH 5.5) consisted of 50 mM HEPES, 2.5 mM L-tyrosine **4** (1.00 g), 10 mM sodium ascorbate **7**, 100 μM $CuSO_4{\cdot}5H_2O$, 1.25 mM PLP and 1.25 mM sodium pyruvate. To initiate the hydroxylation and decarboxylation steps, 10% (v/v) of *Cn*TYR cell lysate and 10% (v/v) of *Ef*TyrDC cell lysate were added to the reaction mixture which was incubated at 25 °C, 250 rpm for 24 h. Then the reaction mixture was adjusted to pH 7.5 with 2.5 M NaOH, and the next steps performed with 10% (v/v) of *Cv*TAm lysate and 50 μg/mL of *Tf*NCS at 37 °C, 250 rpm for 16 h. The product was purified using preparative HPLC (method 4, Vydac^{TM} 218TP1022 (C18, 10 μm, 2.2 cm x 25 cm) preparative column, retention time: 15.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*S*)-**21** as a powder (yield by HPLC (calibration curve) 85% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.4 min, run time: 10 mins, flow rate: 1 mL/min); final isolated yield 0.276 g, 43% (>97% *e.e.*).

Entry 6: The reaction mixture (200 mL, pH 5.5) consisted of 50 mM HEPES, 15 mM *meta*-L-tyrosine **8** (0.544 g), 60 mM sodium ascorbate, 7.5 mM PLP and 7.5 mM sodium pyruvate. To initiate the decarboxylation step, 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture which was incubated at 25 °C, 250 rpm for 16 h. Then, the reaction was adjusted to pH 7.5 with 2.5 M NaOH, and the and the next steps performed with 20% (v/v) of *Cv*TAm and 100 μg/mL of *Tf*NCS at 37 °C, 250 rpm for 24

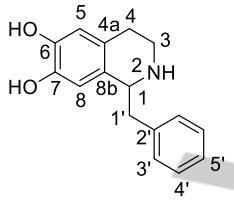
h. Yield by HPLC (calibration curve) 72% (method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.2 min, run time: 10 mins, flow rate: 1 mL/min); The solution was concentrated to 100 mL *in vacuo*, and was extracted with ethyl acetate (7 x 100 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a yellow oil. The residue was resuspended in 20 mL of a 1:1 mixture of HCl (1 M)/dimethyl carbonate (DMC), and the aqueous layer was washed with DMC (3 x 5 mL). The aqueous phase was co-evaporated with methanol at 45 °C to obtain a white solid (*S*)-**27**, as the HCl salt (0.171 g, 39%).

5. KPi reaction to prepare racemic analytical standards

KPi buffer: 300 mM K_2HPO_4 was prepared as solution A and 300 mM KH_2PO_4 was prepared as solution B. Solutions A and B were combined until the mixture was pH 6, giving the KPi buffer (pH 6, 0.3 M).

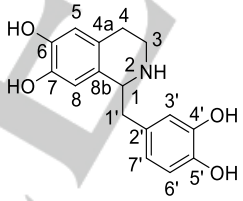
KPi buffer-mediated Pictet-Spengler condensation:

(*rac*)-**20**



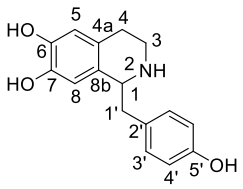
Dopamine **2** (20 mM), sodium ascorbate **7** (80 mM) and phenylacetaldehyde **19** (20 mM) were added to 20 mL of a 1:1 mixture of acetonitrile/ KPi buffer (0.3 M, pH 6). The solution was stirred at 60 °C for 18 h under Ar and then was adjusted to pH 7.5 by adding NaOH (2.5 M). The solution was extracted with ethyl acetate (3 x 30 mL). The organic layer was dried (Na_2SO_4) and evaporated to give a yellow oil. The residue was resuspended in 20 mL of a 1:1 mixture of HCl (1 M)/dimethyl carbonate (DMC), and the aqueous layer was washed with DMC (3 x 5 mL). The aqueous phase was co-evaporated with methanol at 45 °C to obtain an off- white solid (*rac*)-**20**, as the HCl salt (71 mg, 61%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.3 min, run time: 10 mins, flow rate: 1 mL/min) chiral HPLC for comparison to the enzyme product, method 2, Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 12 min and 15 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**20**.

(*rac*)-**21**[2]



Dopamine **2** (20 mM), sodium ascorbate **7** (80 mM) PLP (10 mM) and sodium pyruvate (15 mM) were added to 20 mL of a 1:4 mixture of acetonitrile/ KPi buffer (0.3 M, pH 7.5). 20% (v/v) of *Cv*TAm cell lysate were added to the reaction mixture which was incubated at 37 °C, 250 rpm for 10 h. Then, the solution was adjusted to pH 6 by adding KH_2PO_4 (0.3 M) and was stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 4, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 15.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*rac*)-**21** (16 mg, 28%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.4 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product, method 3, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 80.0 min and 89.0 min, run time: 100 mins, flow rate: 0.2 mL/min). The NMR characterisation data was identical to that of (*S*)-**21**.

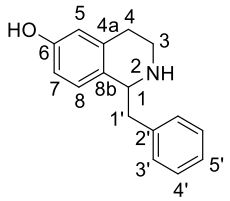
(*rac*)-**1**[3]



Tyramine **6** (20 mM), sodium ascorbate **7** (80 mM), PLP (10 mM) and sodium pyruvate (15 mM) were added to 20 mL of a 1:4 mixture of acetonitrile/ KPi buffer (0.3 M, pH 7.5). 20% (v/v) of *Cv*TAm cell lysate were added to the reaction mixture which was

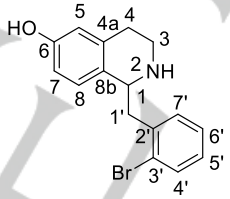
incubated at 37 °C, 250 rpm for 6 h under the Ar. Then, the reaction mixture was adjusted to pH 6 with KH_2PO_4 (0.3 M), and dopamine **2** (10 mM) was added to the solution which was then stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 4, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 15.5 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*rac*)-**1** (24 mg, 44%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 4.8 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product, method 2, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 11.6 min and 14.0 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**1.**

(*rac*)-**23**[3]



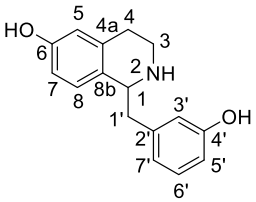
Meta-L-tyrosine **8** (20 mM) and PLP (10 mM) were added to 20 mL of KPi buffer (0.3 M, pH 6). 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture and incubated at 30 °C, 250 rpm for 8 h. Then, acetonitrile (20 mL) and phenylacetaldehyde **19** (15 mM) were added to the solution which was stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 5, Vydac™ 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 12.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give a white powder (*rac*)-**23** (41 mg, 43%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.9 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product method 2, Supelco Astec Chirobiotic™ T2 column (25 cm × 4.6 mm), retention time: 18.0 min and 20.0 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**23.**

(*rac*)-**25**



Meta-L-tyrosine **8** (20 mM) and PLP (10 mM) were added to 20 mL of KPi buffer (0.3 M, pH 6). 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture and incubated at 30 °C, 250 rpm for 8 h. Then, acetonitrile (20 mL) and 2-(2-bromophenyl)acetaldehyde **24** (15 mM) were added to the solution which was stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 5, Supelco™ Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column, retention time: 14.0 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give a brown powder (*rac*)-**25** (32 mg, 25%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 6.0 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product method 2, Supeclo Astec Chirobiotic™ T2 column (25 cm × 4.6 mm), retention time: 18.0 min and 22.0 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**25.**

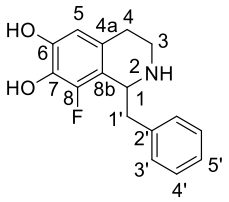
(*rac*)-**27**[2]



Meta-L-tyrosine **8** (20 mM) and PLP (10 mM) were added to 40 mL of KPi buffer (0.3 M, pH 6). 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture and incubated at 30 °C, 250 rpm for 8 h. Then, 20% (v/v) acetonitrile (20 mL), sodium ascorbate **7** (80 mM) and sodium pyruvate (10 mM) were added to the solution which was reacted with 20% (v/v) of *Cv*TAm cell lysate at 37 °C, 250 rpm for 6 h and then the solution was stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 4, VydacTM 218TP1022 (C18, 10 µm, 2.2 cm x 25 cm) preparative column, retention time: 17.1 min, run time:

28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*rac*)-**27** (37 mg, 36%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.2 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product method 3, Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 14.0 min and 17.2 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**27.**

(*rac*)-**29**



3-F-L-Tyrosine **9** (20 mM) and PLP (10 mM) were added to 20 mL of KPi buffer (0.3 M, pH 6). 20% (v/v) of *Ef*TyrDC cell lysate was added to the reaction mixture which was incubated at 30 °C, 250 rpm for 8 h. Then, CuSO4 (100 µM), sodium ascorbate **7** (80 mM) and 20% (v/v) of *Cn*TYR cell lysate was added to the solution and incubated at 25 °C, 250 rpm for 16 h. After, acetonitrile (20 mL) and phenylacetaldehyde **19** (15 mM) were added to the solution. It was stirred at 60 °C for 18 h under Ar. The product was purified using preparative HPLC (method 4, Supelco™ Discovery BIO wide pore (C18, 10 µm, 2.12 cm x 25 cm) preparative column, retention time: 18.6 min, run time: 28 mins, flow rate: 8 mL/min). Fractions containing the desired product were freeze-dried to give (*rac*)-**29** (16 mg, 15%) (analytical HPLC, method 1, ACE 5 C18 column (150 × 4.6 mm), retention time: 5.5 min, run time: 10 mins, flow rate: 1 mL/min; chiral HPLC for comparison to the enzyme product, method 2, Supelco Astec Chirobiotic™ T column (25 cm × 4.6 mm), retention time: 12.0 min and 15.0 min, run time: 40 mins, flow rate: 1 mL/min). The NMR characterisation data was identical to that of (*S*)-**29.**

6. Analytical HPLC results

6.1 Achiral analytical HPLC results for single *Cn*TYR reaction products

Achiral separation was achieved using **Analytical HPLC method 1**(SI chapter 3).

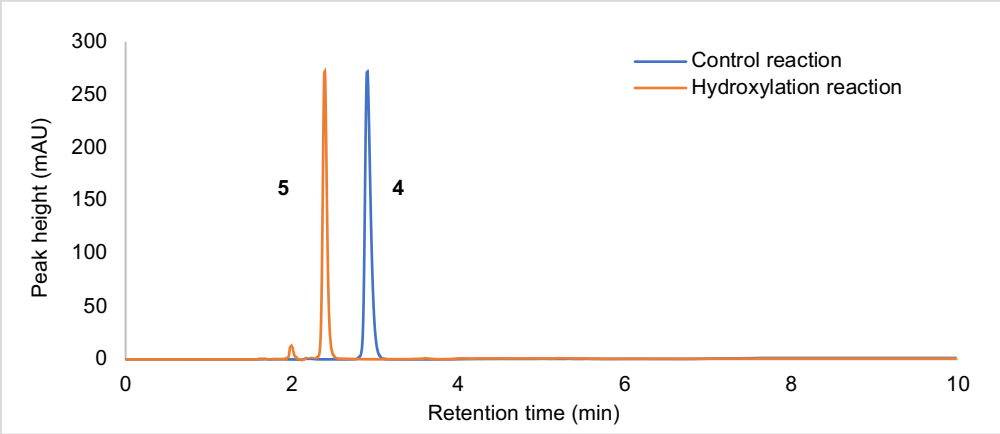


***Figure S12*. Achiral analytical HPLC for the single *Cn*TYR reaction with L-tyrosine 4**

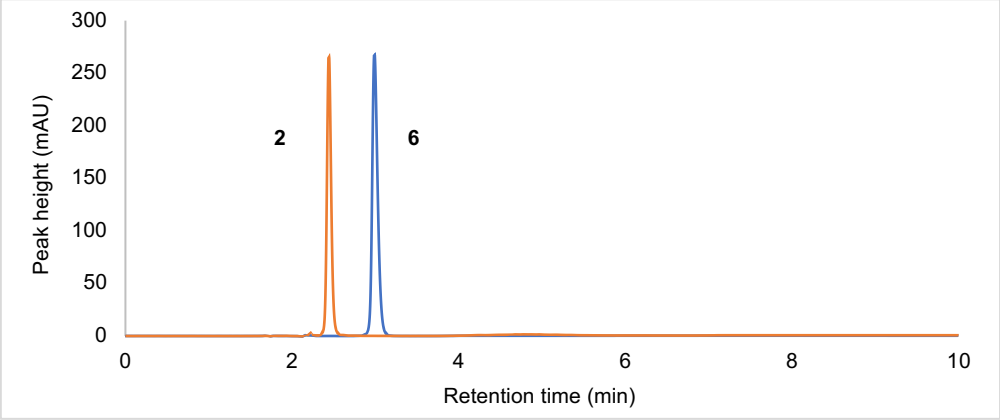


Figure S13. **Achiral analytical HPLC result for the single *Cn*TYR reaction with tyramine 6**

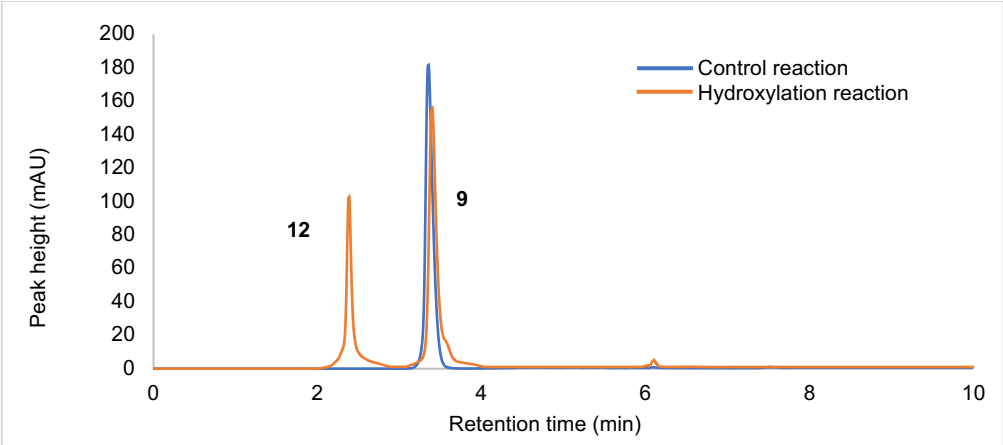


Figure S14. **Achiral analytical HPLC result for the single *Cn*TYR reaction with 3-F-L-tyrosine 9**

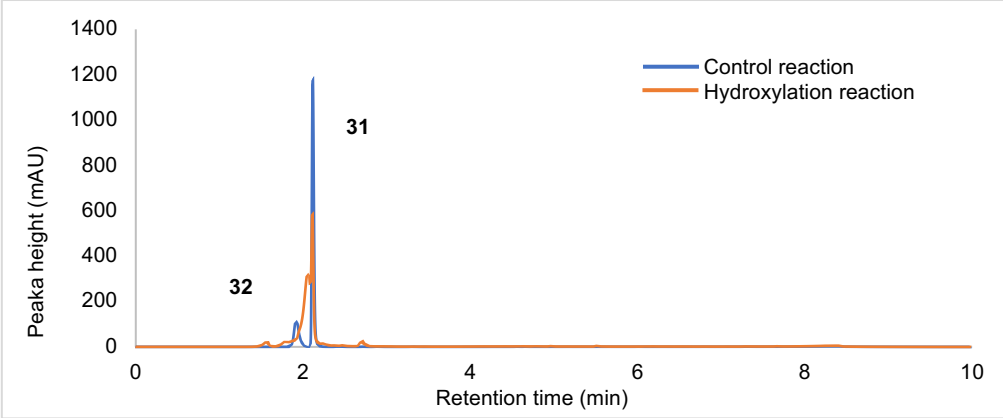
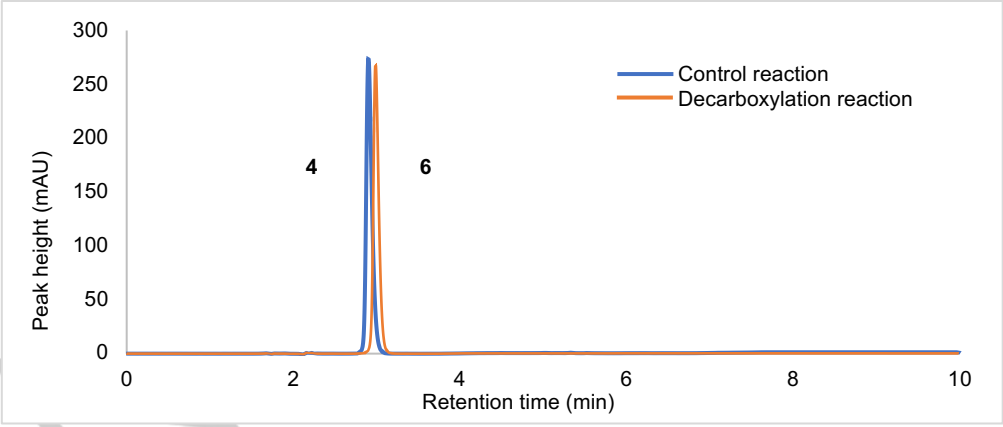


Figure S15. **Achiral analytical HPLC result for the single *Cn*TYR reaction with (*rac*)-octopamine (*rac*)-30**

6.2 Achiral analytical HPLC results for single *Ef*TyrDC reaction products

Achiral separation was achieved using **Analytical HPLC method 1** (SI chapter 3).



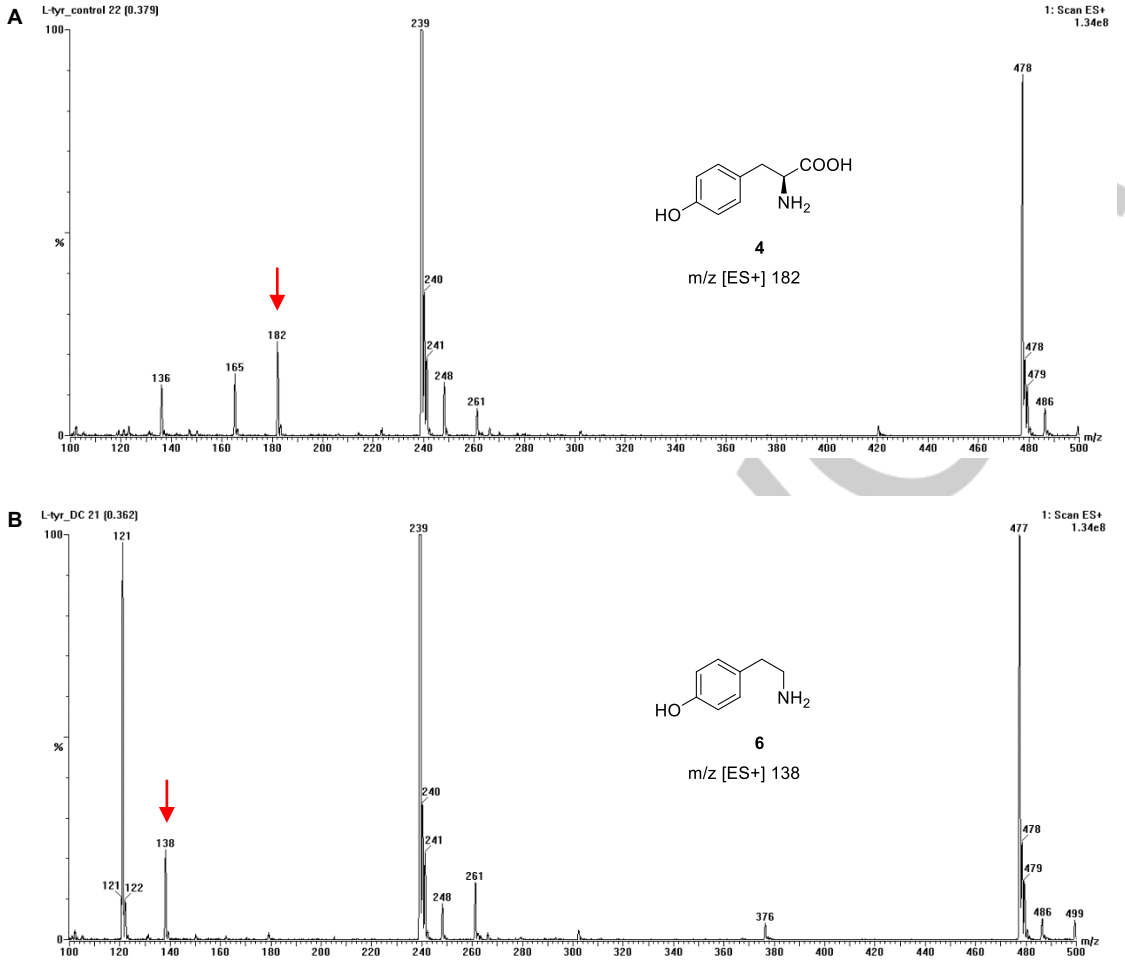
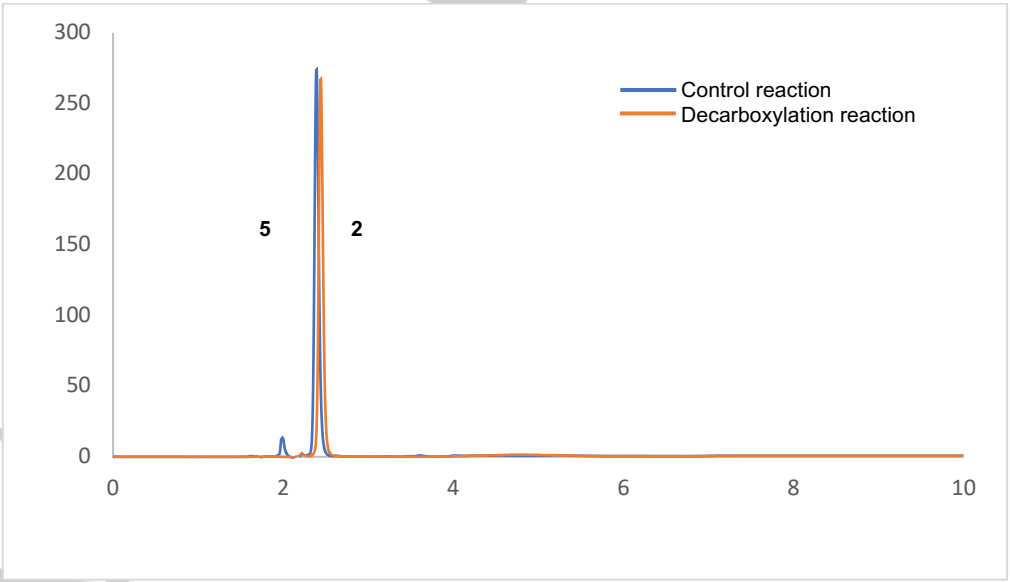


***Figure S16*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with L-tyrosine 4.** To confirm yields, the reaction mixtures both before (A) and after the decarboxylation reactions (B) were analysed by LC-MS and compared to the standards. For the high yielding reactions there was no peak corresponding to the starting material as shown (note that the signal at *m/z* 239 is due to the buffer HEPES).



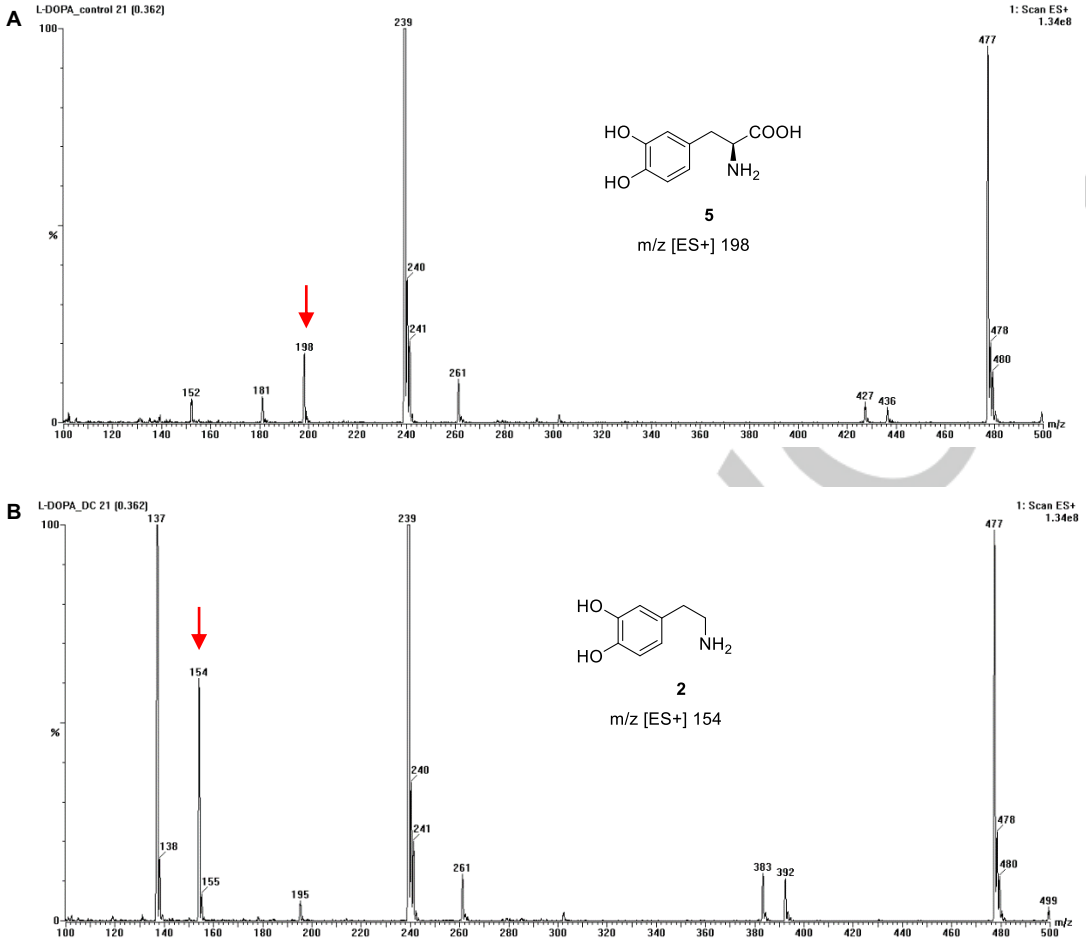


***Figure S17*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with L-DOPA 5 (peak overlap between 2 and 5).** To confirm yields, the reaction mixtures both before (A) and after the decarboxylation reactions (B) were further analysed by LC-MS and compared to the standards. For the high yielding reactions there was no peak corresponding to the starting material as shown (note that the signal at *m/z* 239 is due to the buffer HEPES).

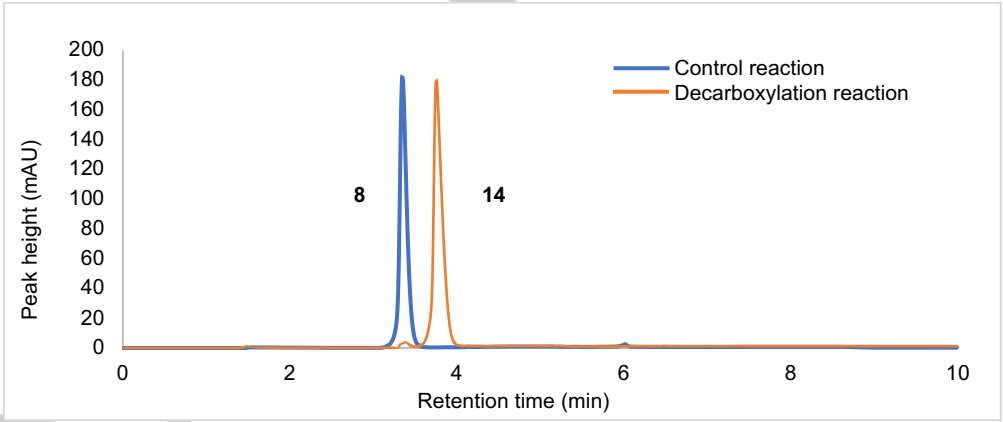
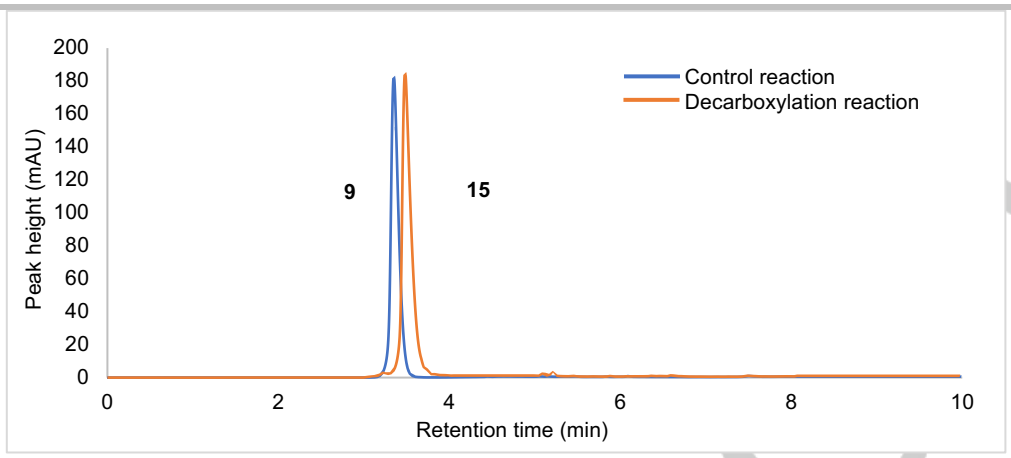


***Figure S18*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with *meta*-L-tyrosine 8**



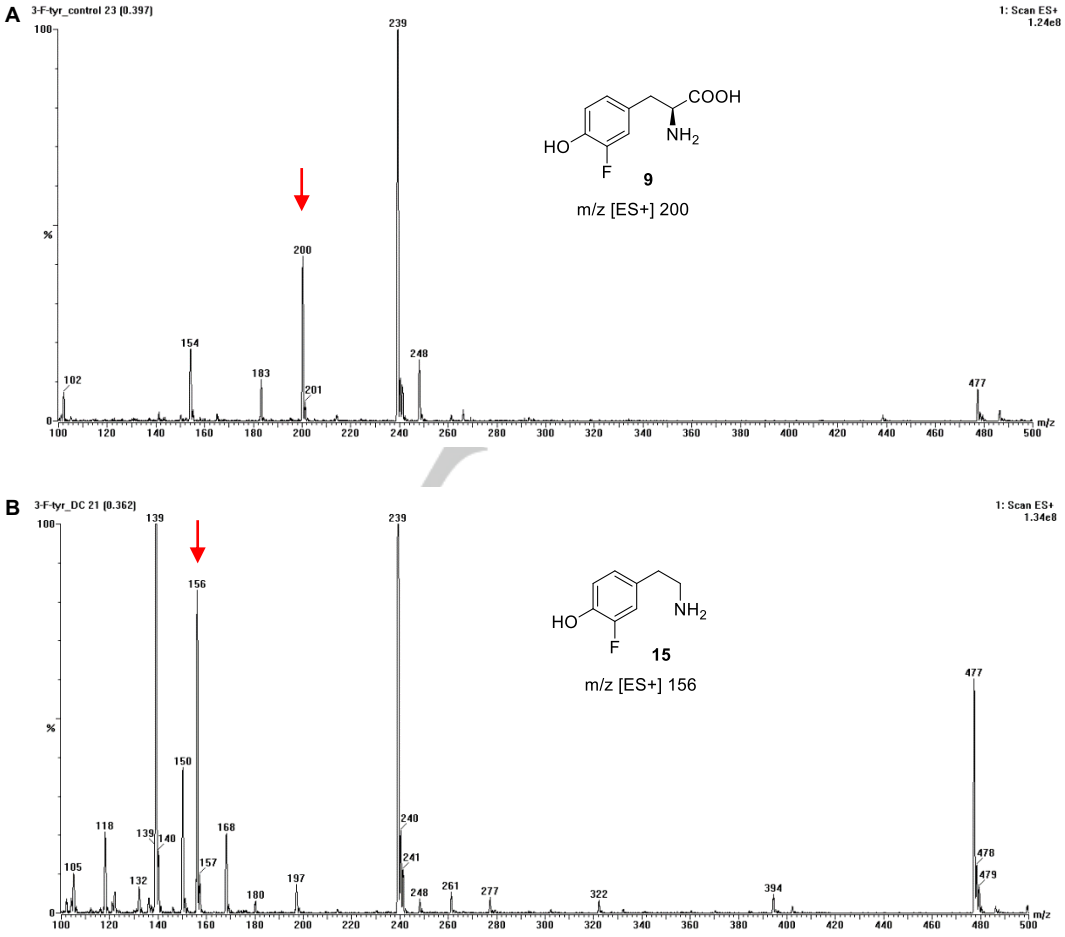


***Figure S19*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with 3-F-L-tyrosine 9** (To confirm yields, the reaction mixtures both before (A) and after the decarboxylation reactions (B) were further analysed by LC-MS and compared to the standards. For the high yielding reactions there was no peak corresponding to the starting material as shown (note that the signal at *m/z* 239 is due to the buffer HEPES).

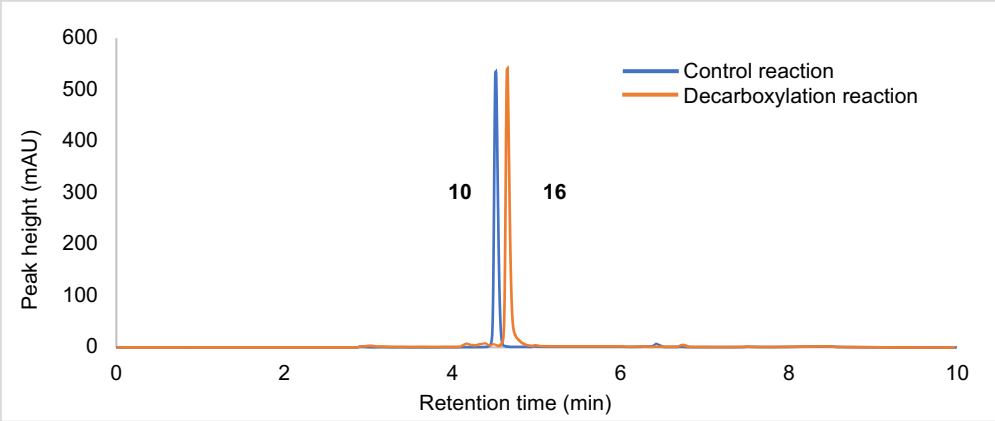


***Figure S20*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with 3-Cl-L-tyrosine 10**

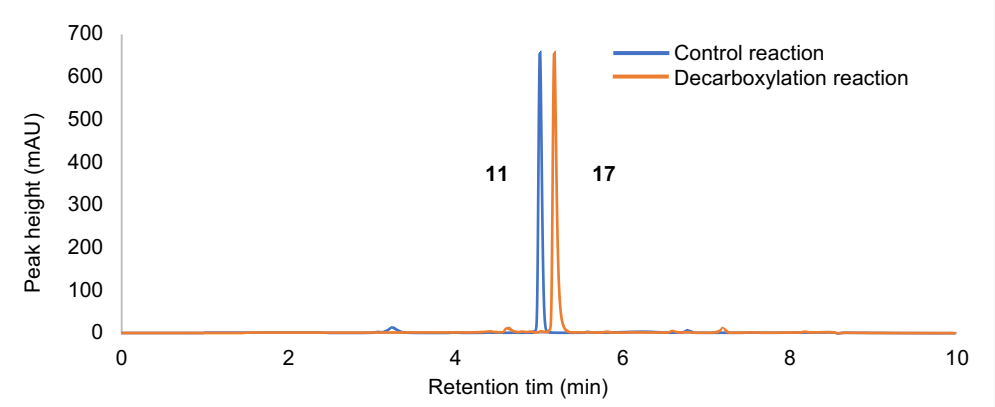


***Figure S21*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with 3-I-L-tyrosine 11**

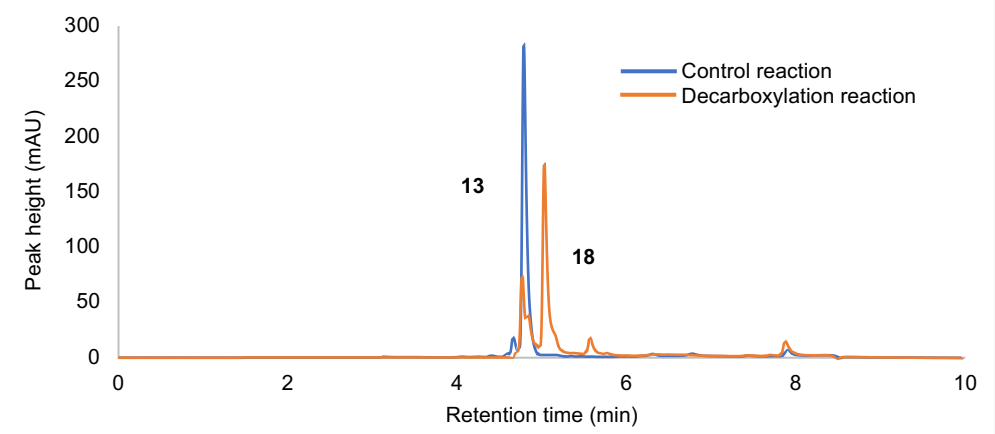


***Figure S22*. Achiral analytical HPLC result for the single *Ef*TyrDC reaction with *para*-Br-*meta*-L-tyrosine 13**

6.3 Achiral analytical HPLC results for *Cn*TYR + *Ef*TyrDC reaction products

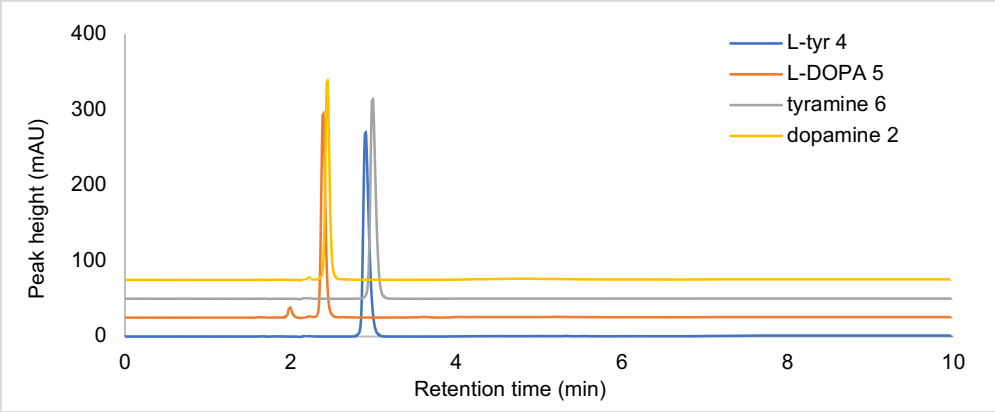


Figure S23. **Analytical HPLC result for the *Cn*TYR + *Ef*TyrDC reaction with L-tyrosine 4**

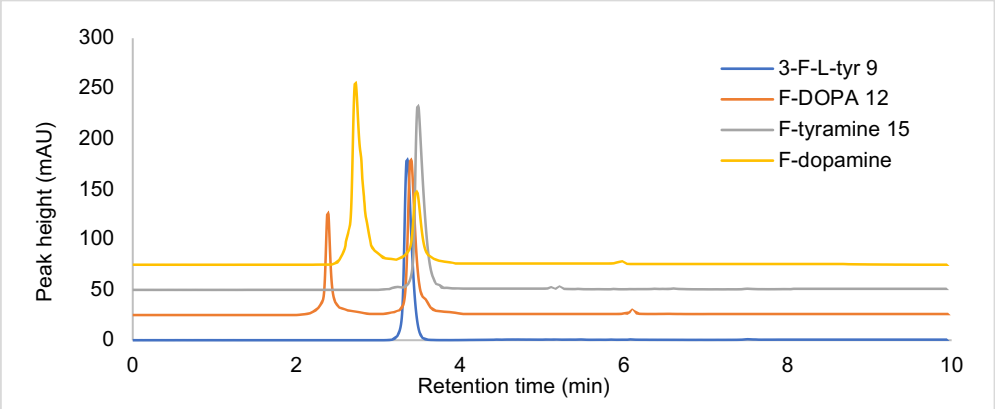
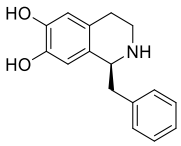


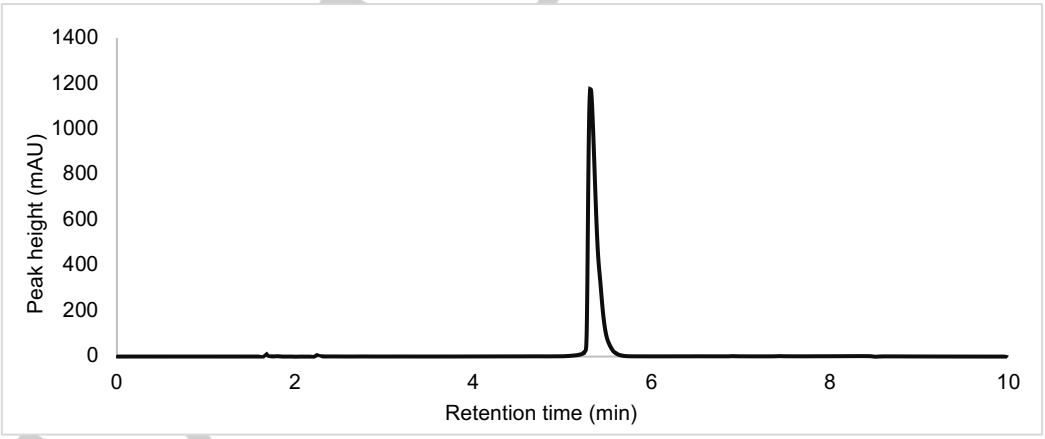
Figure S24. **Analytical HPLC result for the *Cn*TYR + *Ef*TyrDC reaction with 3-F-L-tyrosine 9**

6.4 Achiral analytical HPLC results for cascade reaction products

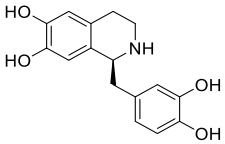
Achiral separation was achieved using **Analytical HPLC method 1**(SI chapter 3).

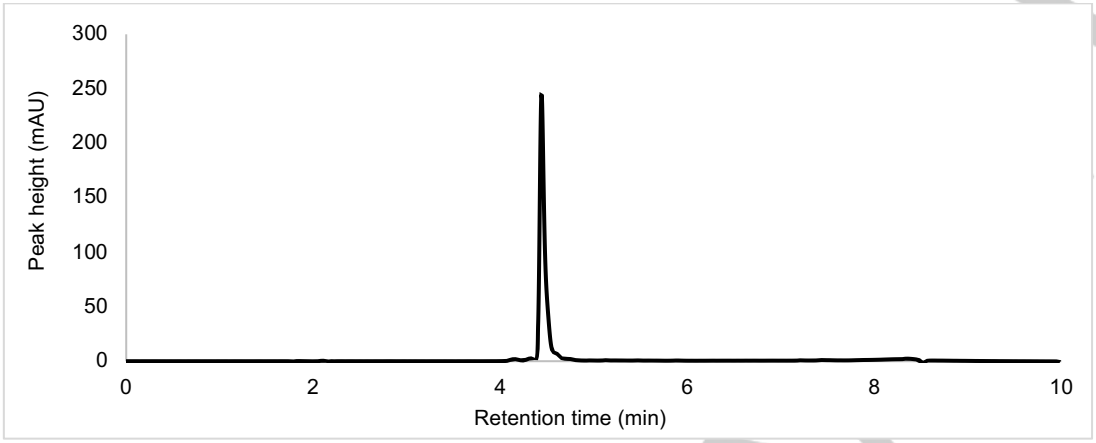
(*S*)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*S*)-**20**



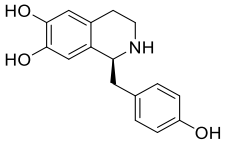


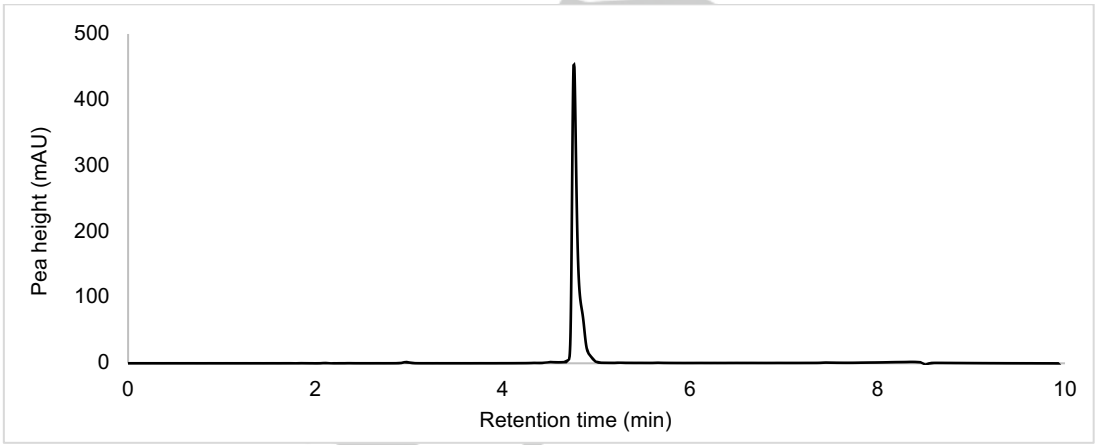
(*S*)-Norlaudanosoline (*S*)-**21**[2]



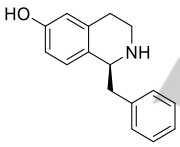


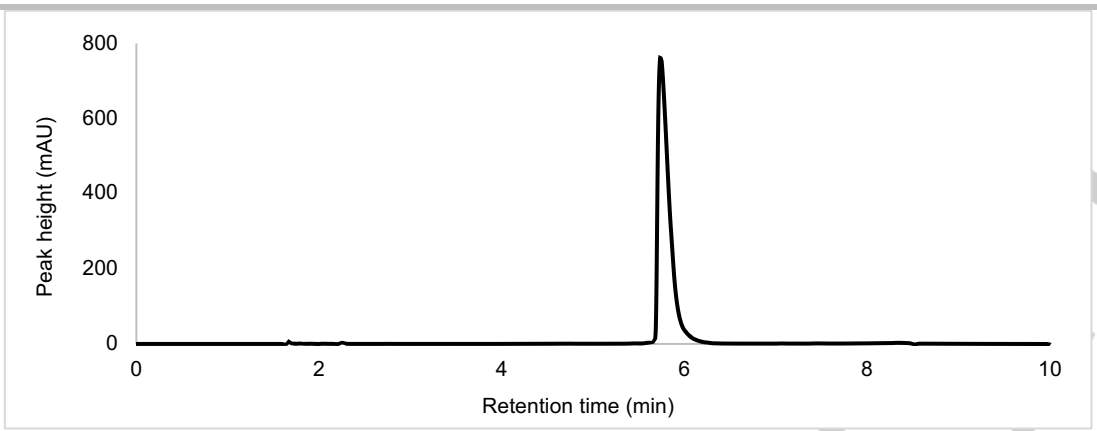
(*S*)-Norcoclaurine (*S*)-**1**[3]



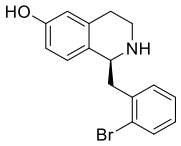


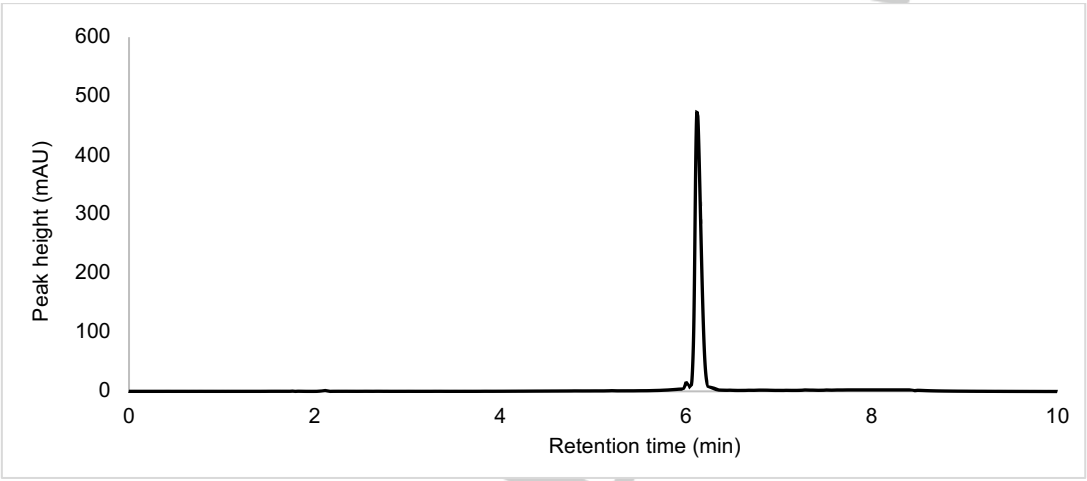
(*S*)-1-Benzyl-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**23**[3]



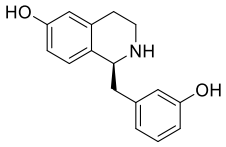


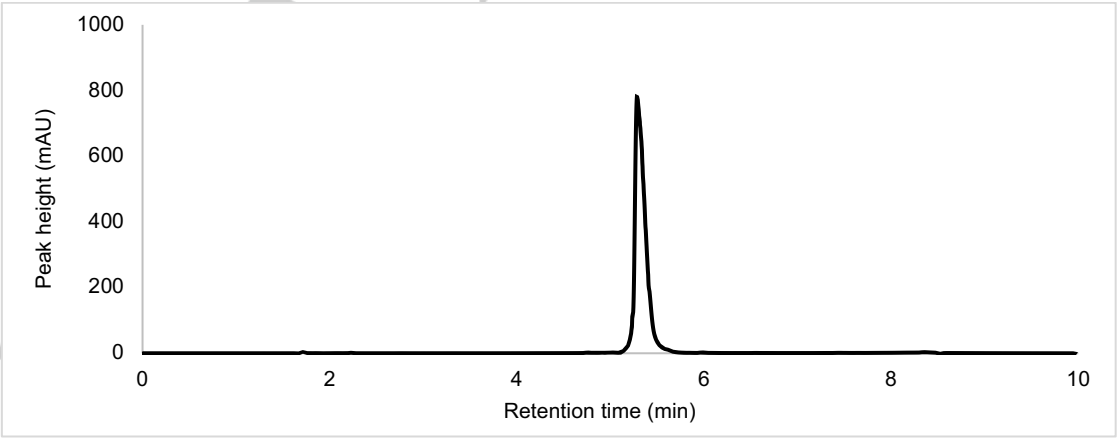
(*S*)-1-(2-Bromobenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**25**



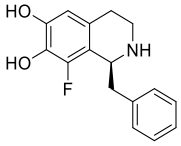


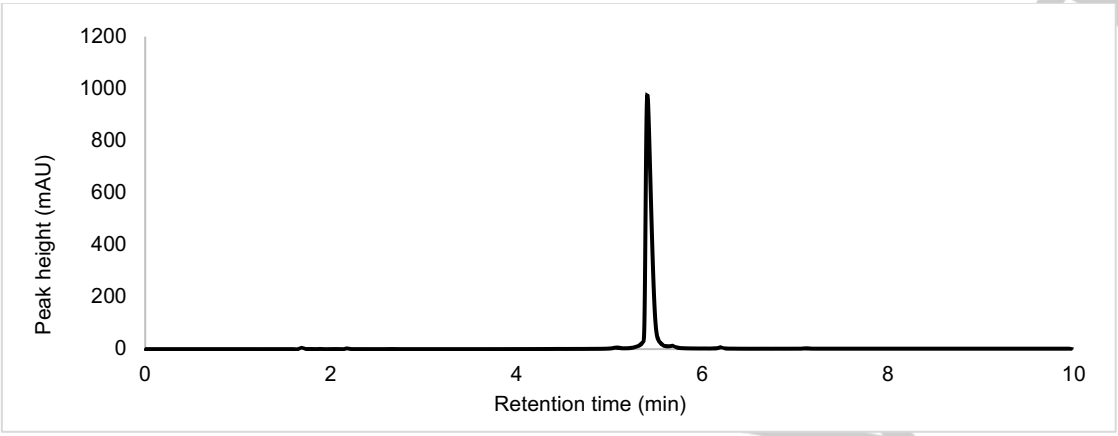
(*S*)-1-(3-Hydroxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**27**[2]



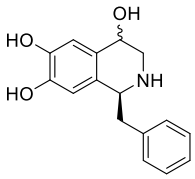


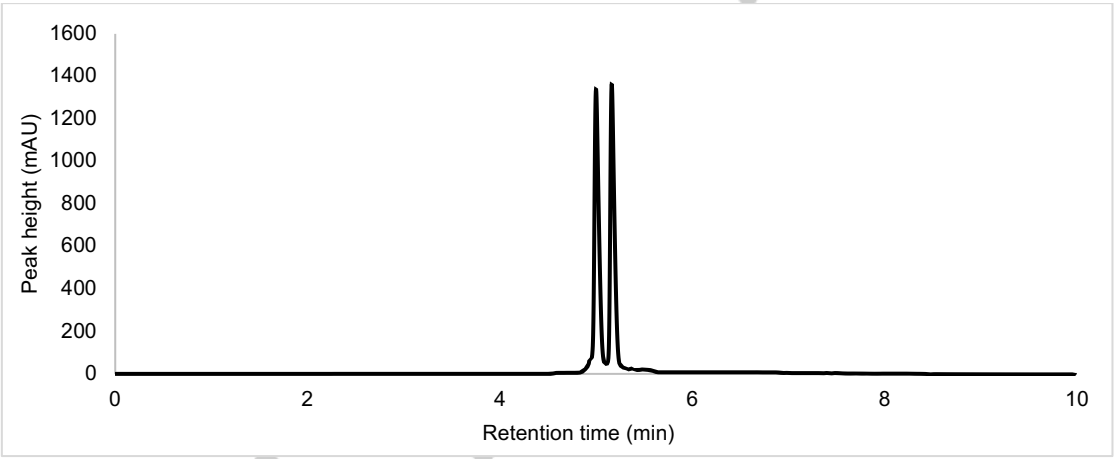
(*S*)-1-Benzyl-8-fluoro-1,2,3,4-tetrahydroisoquinoline-6,7-diol (*S*)-**29**





(1*S, 4RS*)-1-Benzyl-1,2,3,4-tetrahydroisoquinoline-4,6,7-triol (1*S*,4*RS*)-**32**

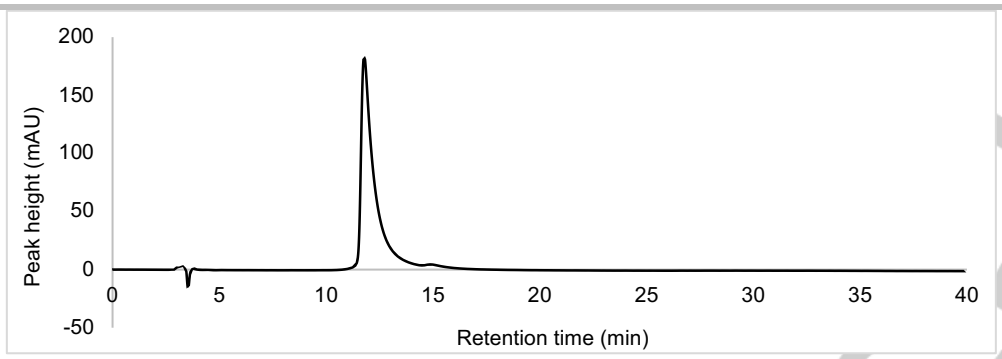


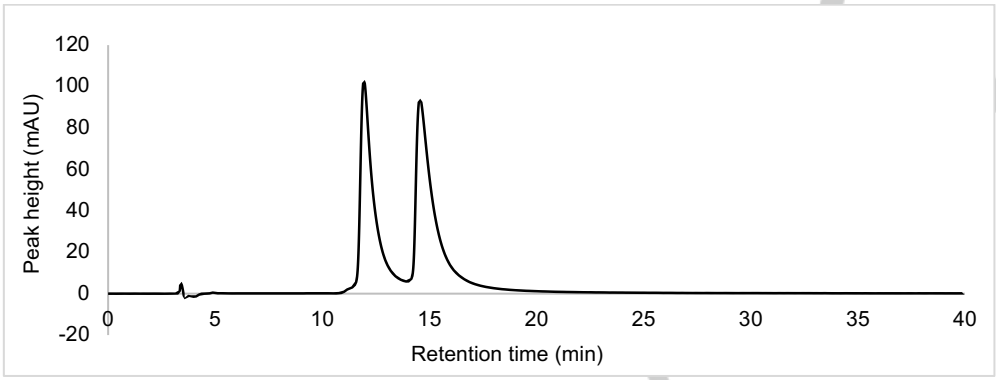


6.5 Chiral analytical HPLC traces for cascade reaction products

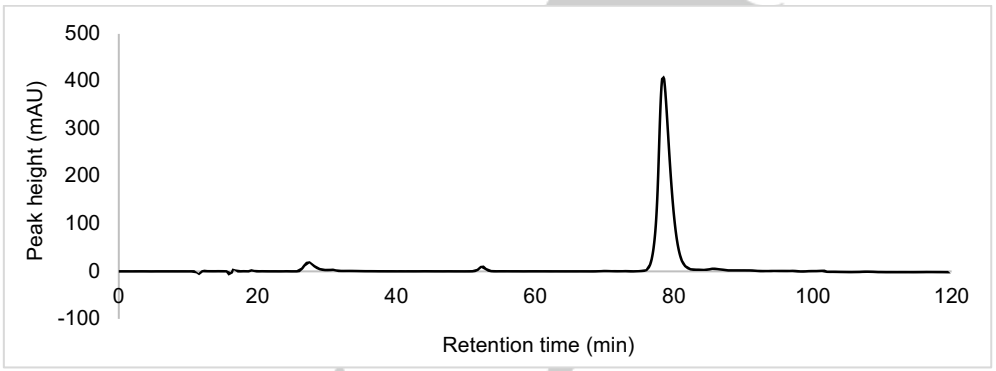
Chiral separation was achieved using **Analytical HPLC method 2 or 3** (SI chapter 3).

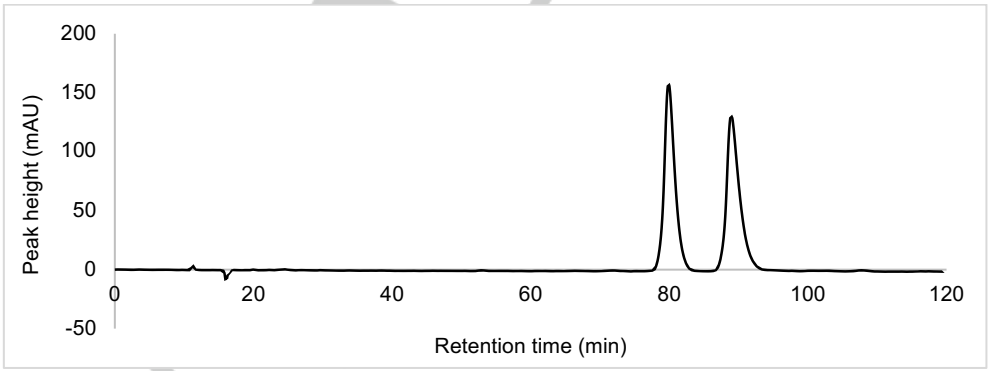
(*S*)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*S*)-**20** and (*rac*)-**20 (Method 2, T column)**



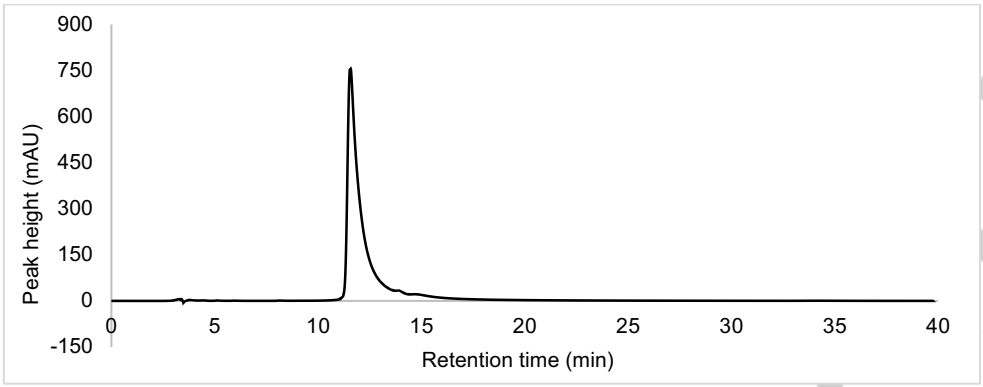


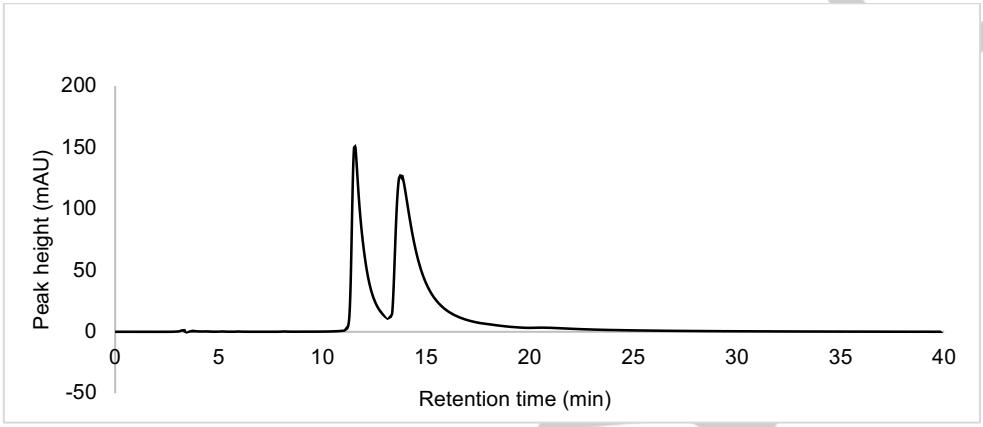
(*S*)-Norlaudanosoline (*S*)-**21** and (*rac*)-**21**[2] **(Method 3, T column)**



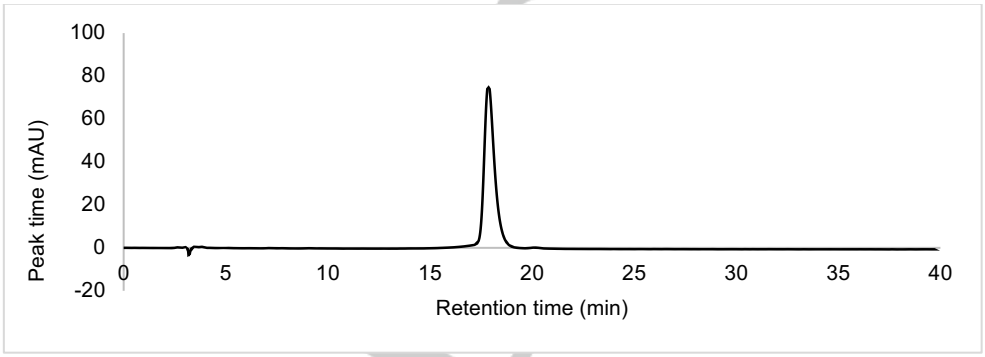


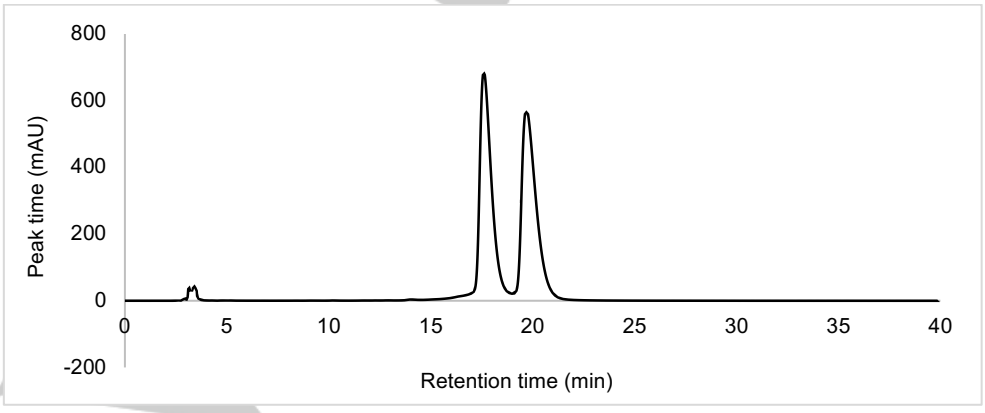
(*S*)- Norcoclaurine (*S*)-**1** and (*rac*)-**1**[3] **(Method 2, T column)**



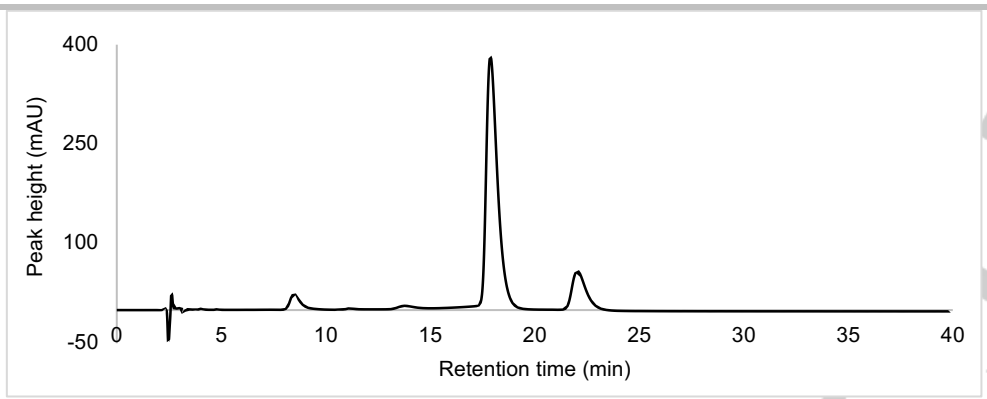


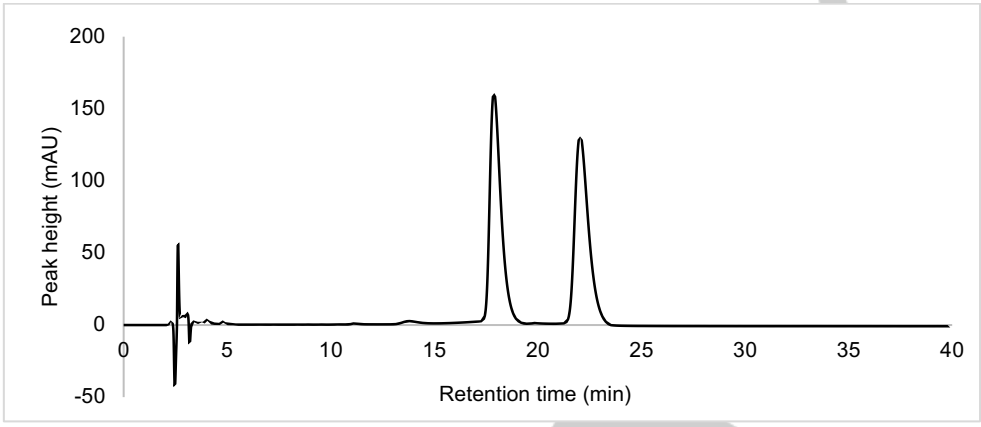
(*S*)-1-Benzyl-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**23** and (*rac*)-**23**[3] **(Method 2, T2 column)**



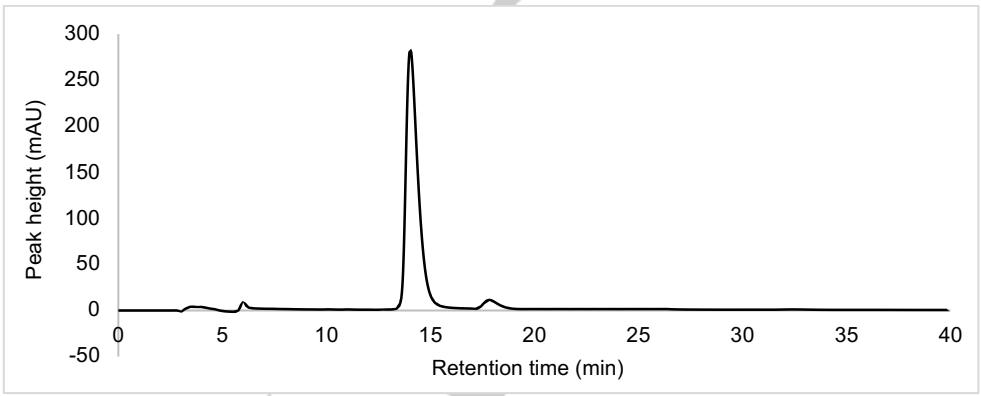


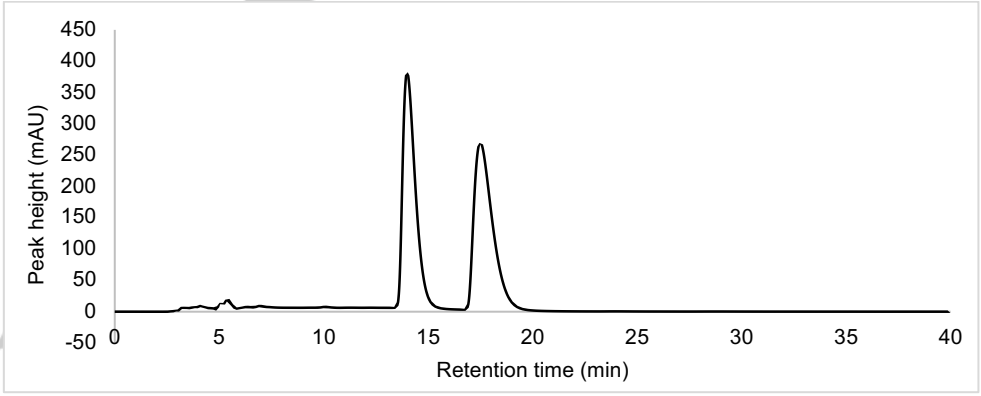
(*S*)-1-(2-Bromobenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**25** and (*rac*)-**25 (Method 2, T2 column)**



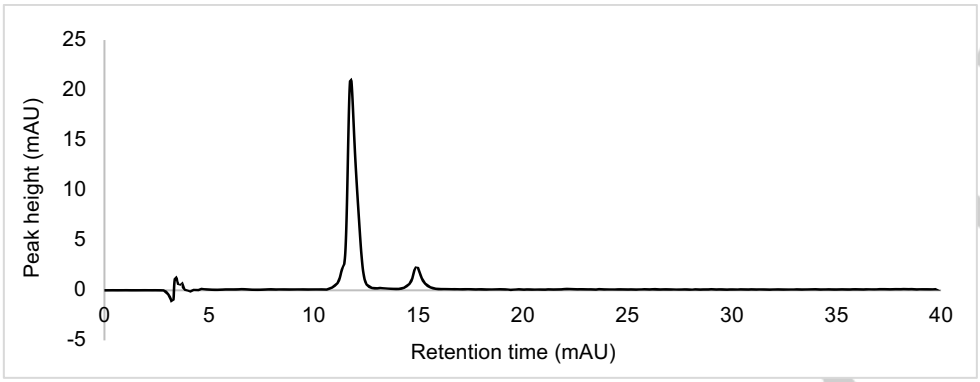


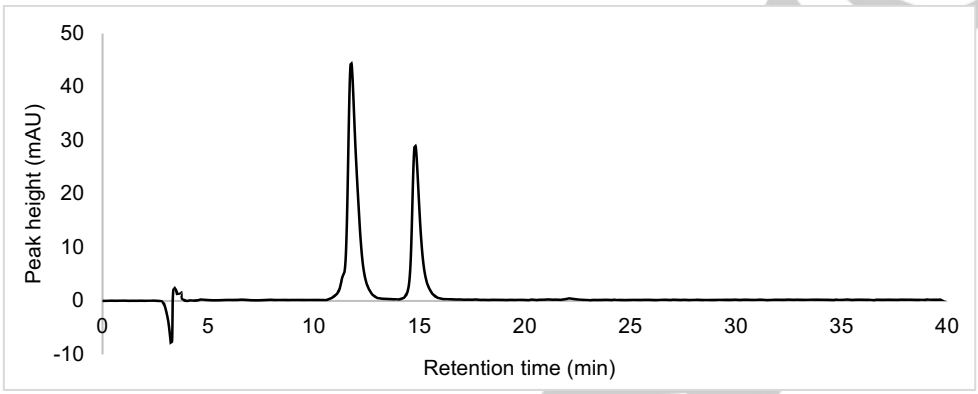
(*S*)-1-(3-Hydroxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**27** and (*rac*)-**27**[2] **(Method 2, T column)**





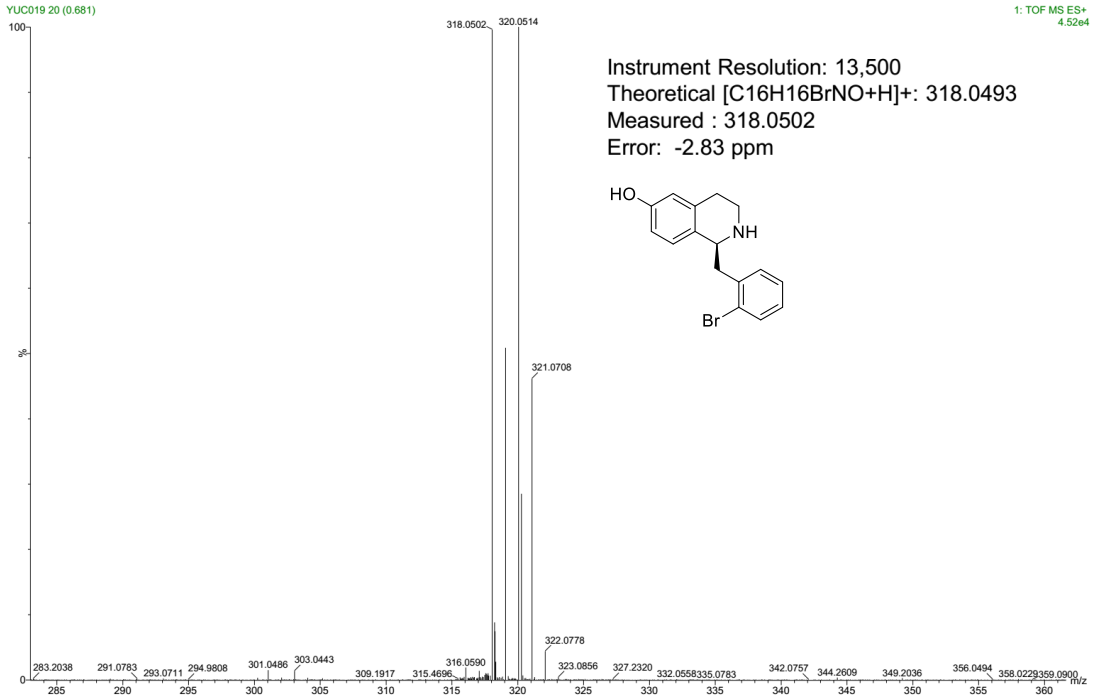
(*S*)-1-Benzyl-8-fluoro-1,2,3,4-tetrahydroisoquinoline-6,7-diol (*S*)-**29** and (*rac*)-**29 (Method 2, T column)**



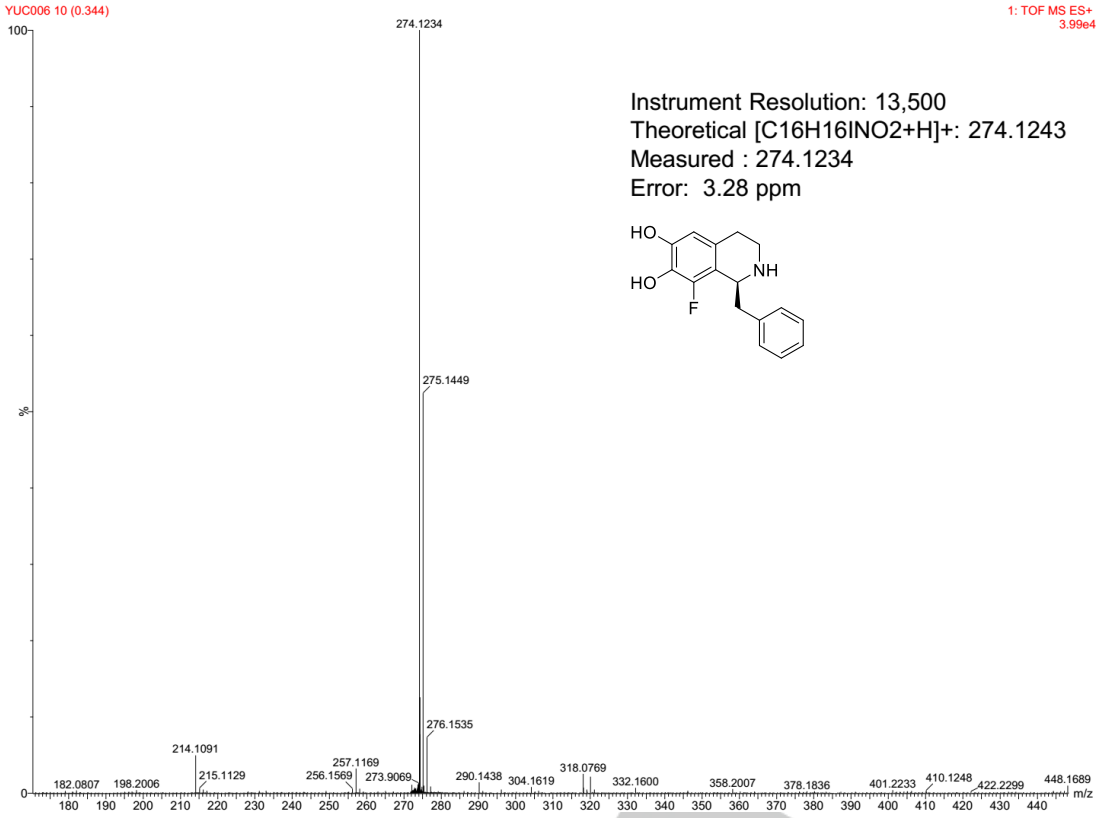


7. Accurate mass results

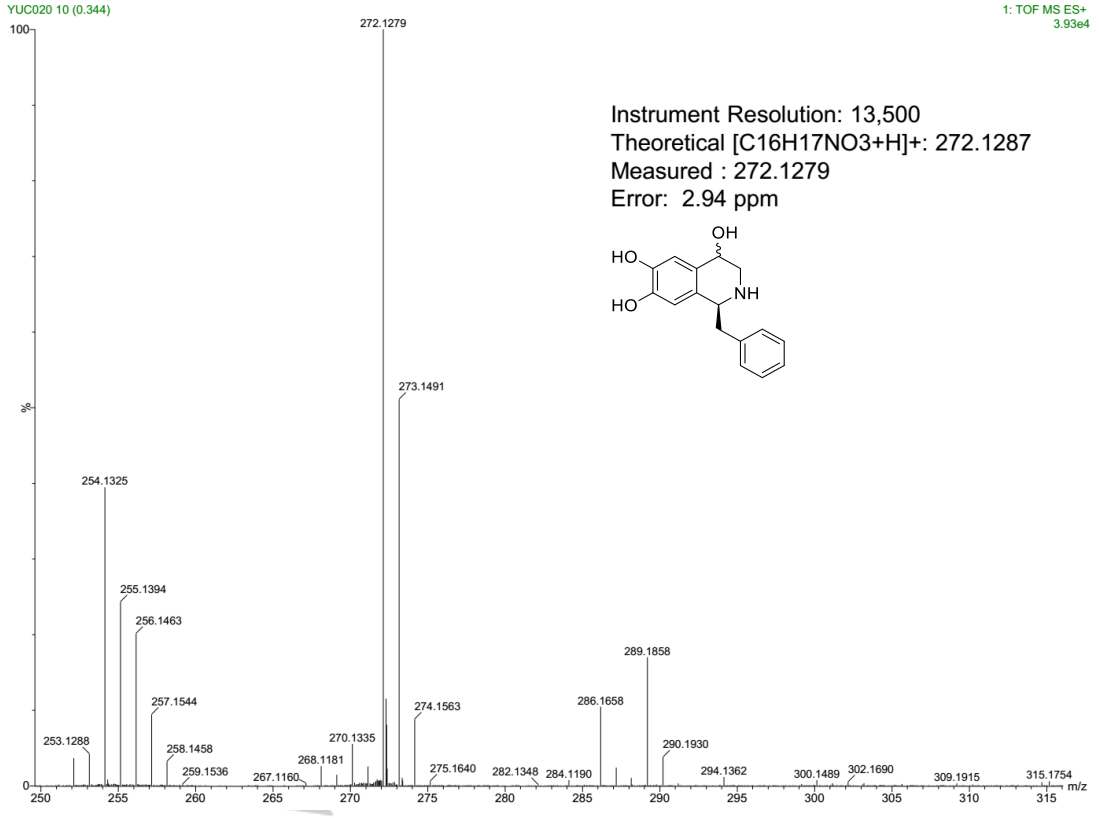
(*S*)-1-(4-Bromobenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**25**



(*S*)-1-Benzyl-8-fluoro-1,2,3,4-tetrahydroisoquinoline-6,7-diol (*S*)-**29**

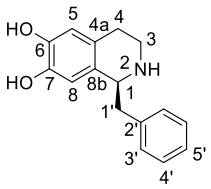


(1*S*, 4*RS*)-1-Benzyl-1,2,3,4-tetrahydroisoquinoline-4,6,7-triol (1*S*, 4*RS*)-**32**

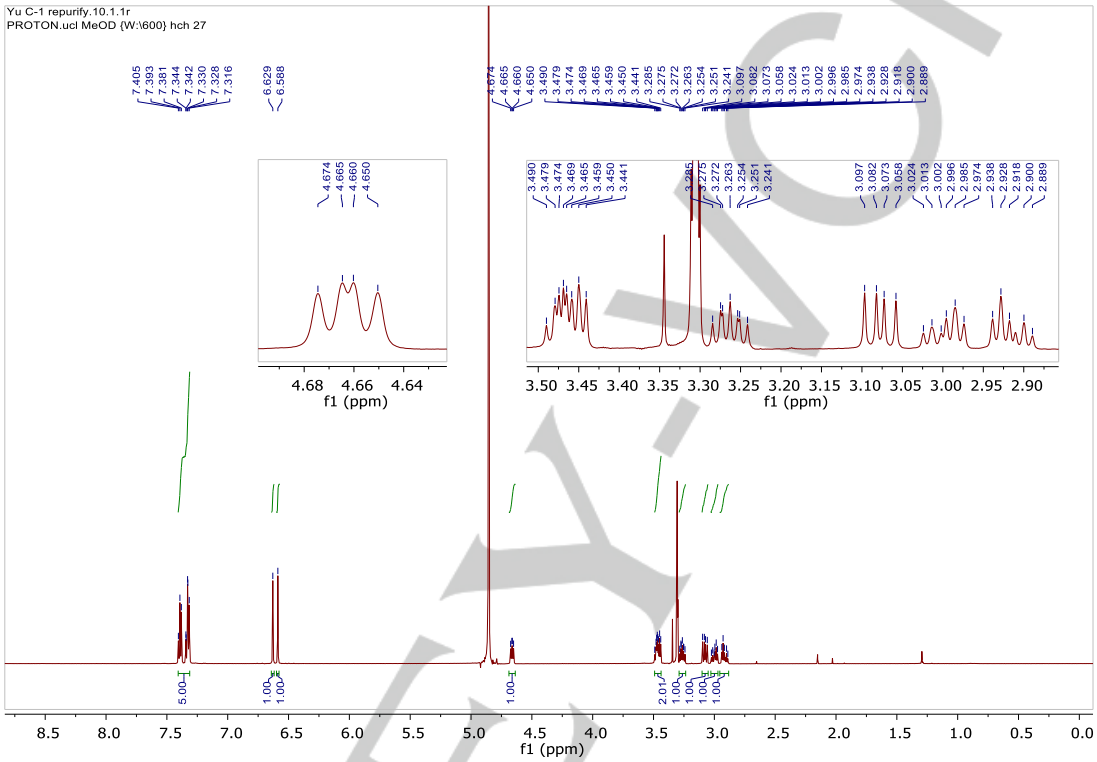


8. NMR spectroscopic data for cascade products

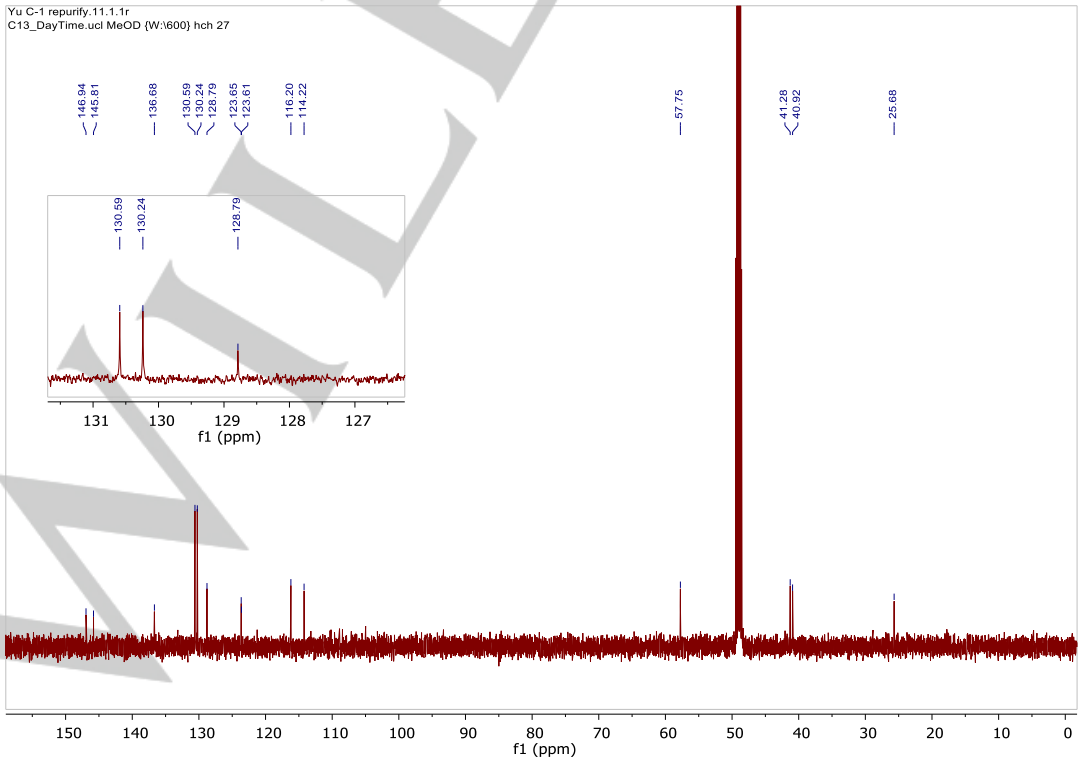
Entry 1: (*S*)-1-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (*S*)-**20**



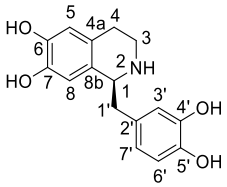
^{1}H NMR (600 MHz; CD_3OD)



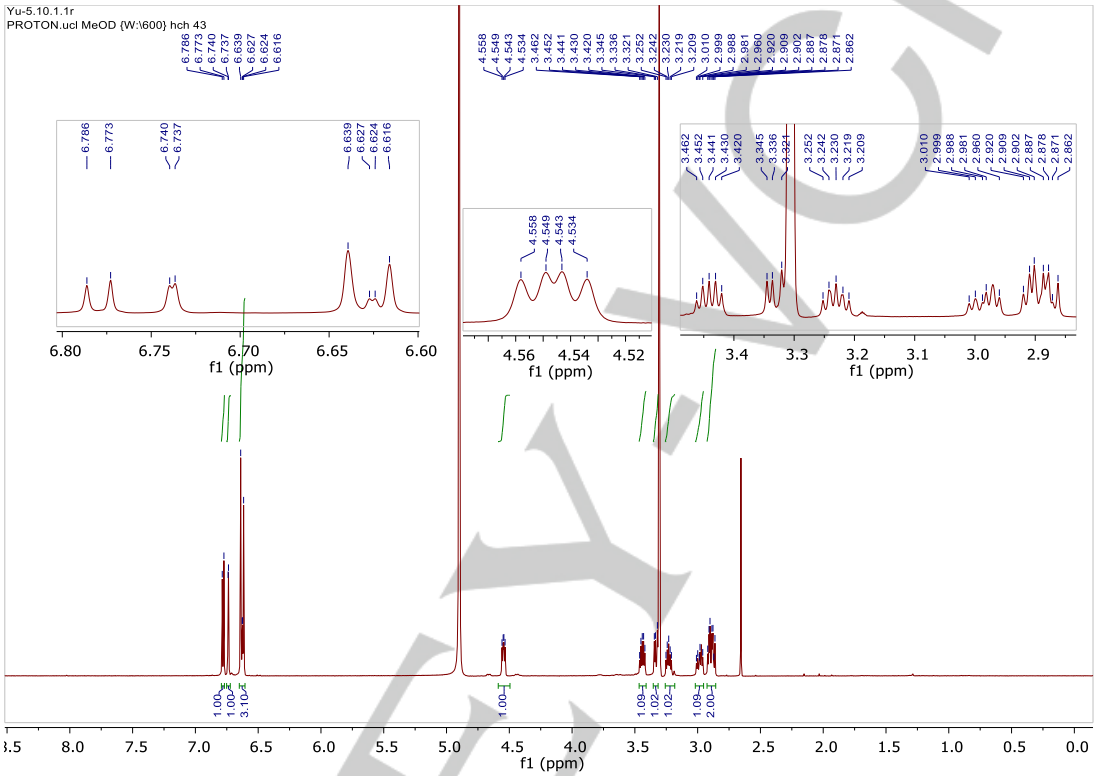
^{13}C NMR (151 MHz; CD_3OD)



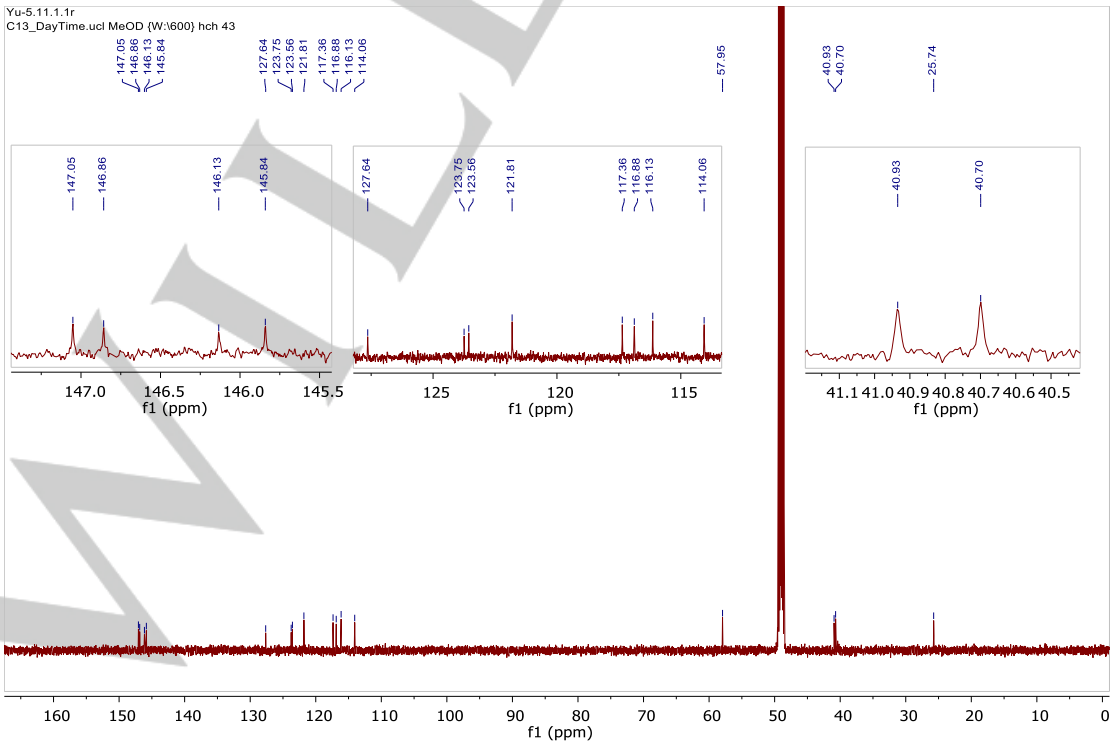
Entry 2: (*S*)-Norlaudanosoline (*S*)-**21**[2]



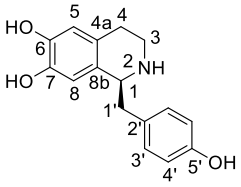
^{1}H NMR (600 MHz; CD_3OD)



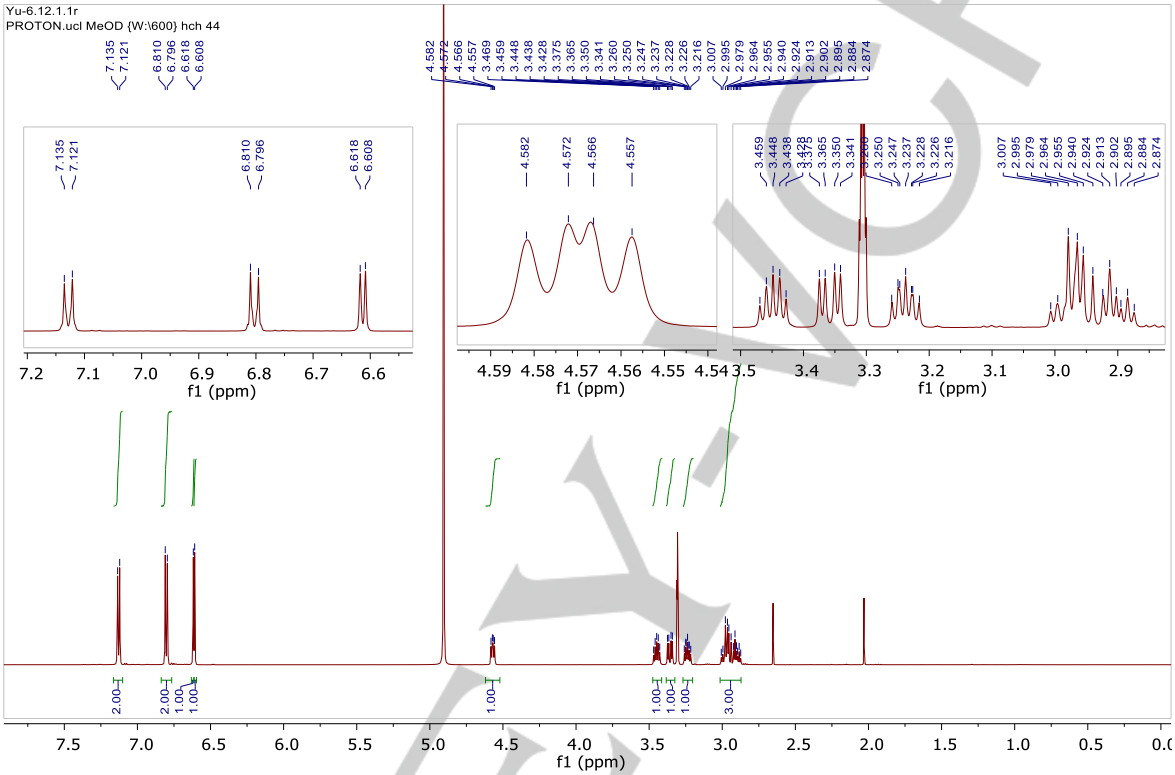
^{13}C NMR (151 MHz; CD_3OD)



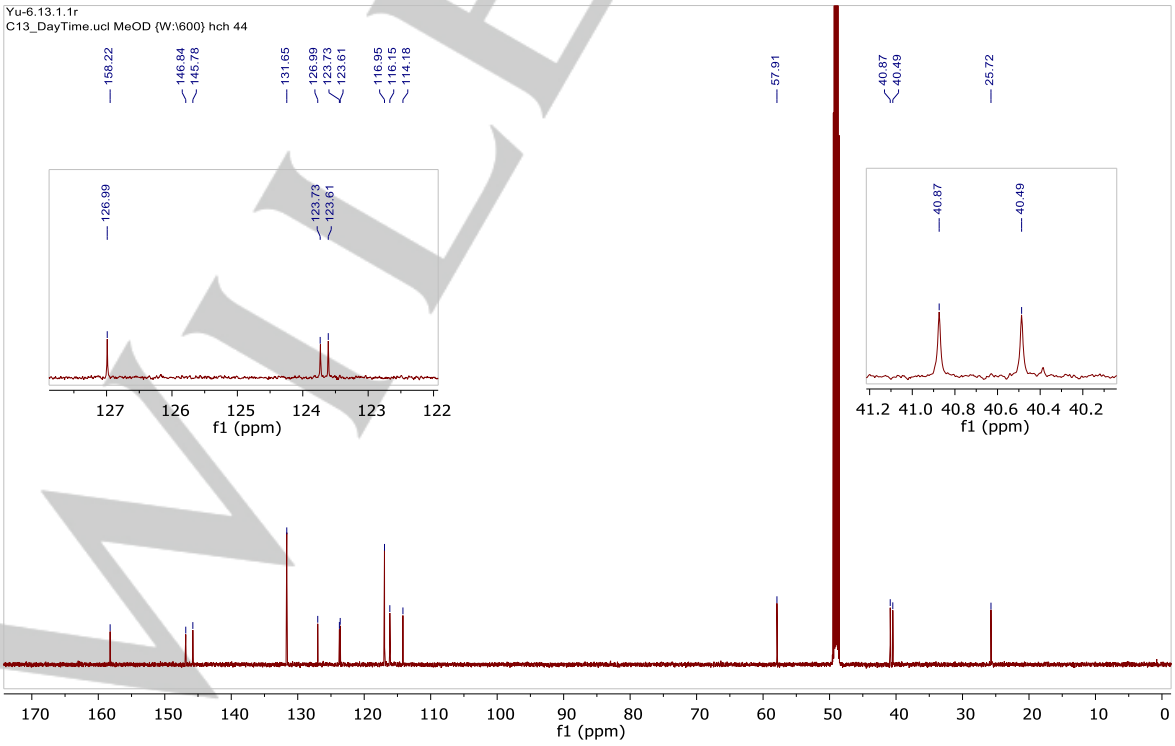
Entry 3: (*S*)-Norcoclaurine (*S*)-**1**[3]



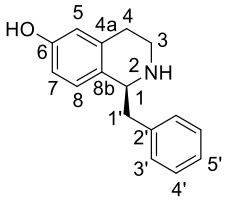
^{1}H NMR (600 MHz; CD_3OD)



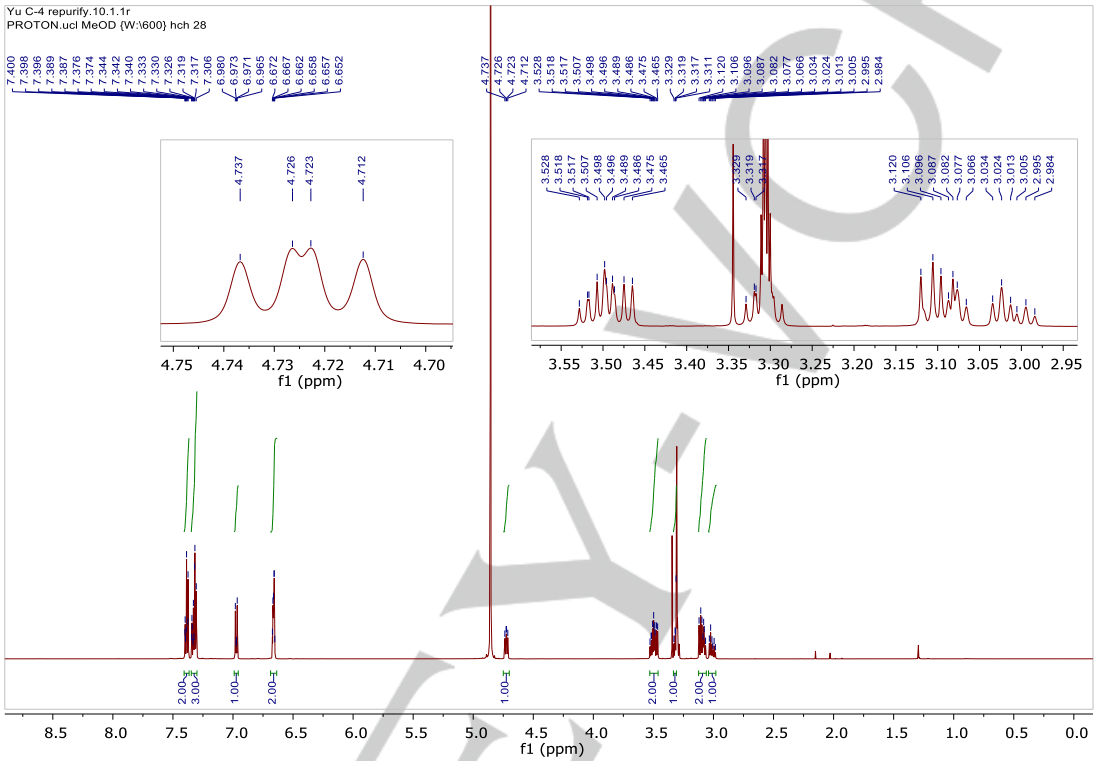
^{13}C NMR (151 MHz; CD_3OD)



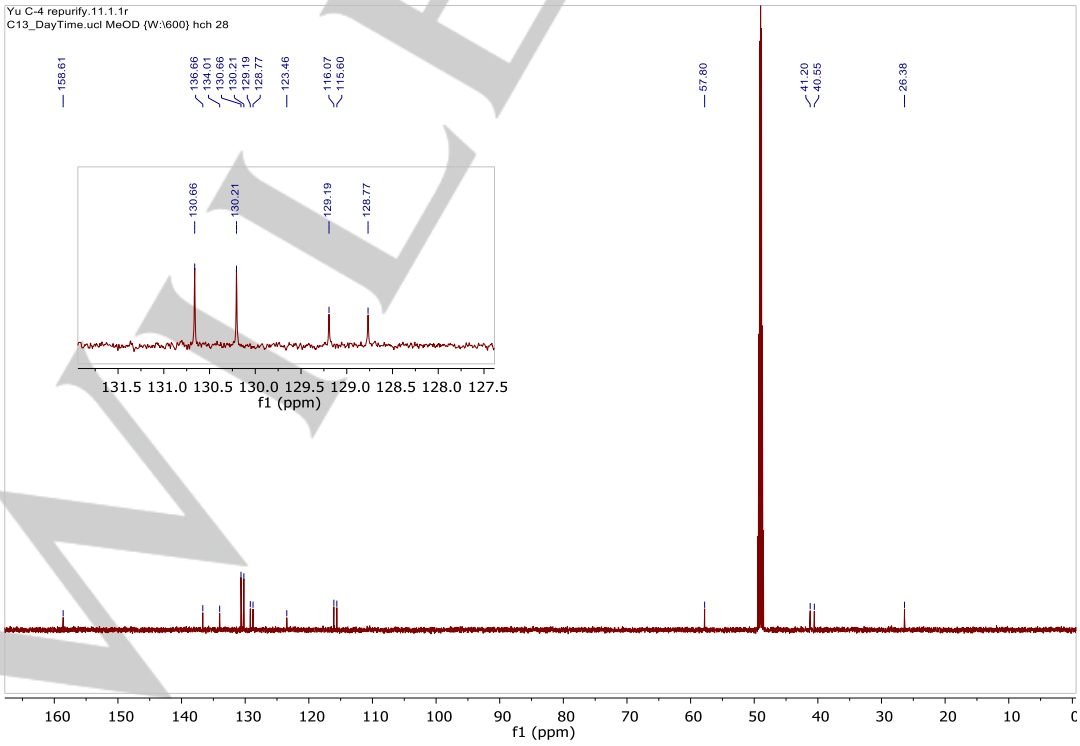
Entry 4: (*S*)-1-Benzy-1,2,3,4- tetrahydroisoquinolin -6-ol (*S*)-**23**[3]



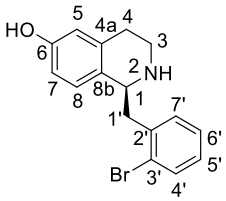
^{1}H NMR (600 MHz; CD_3OD)



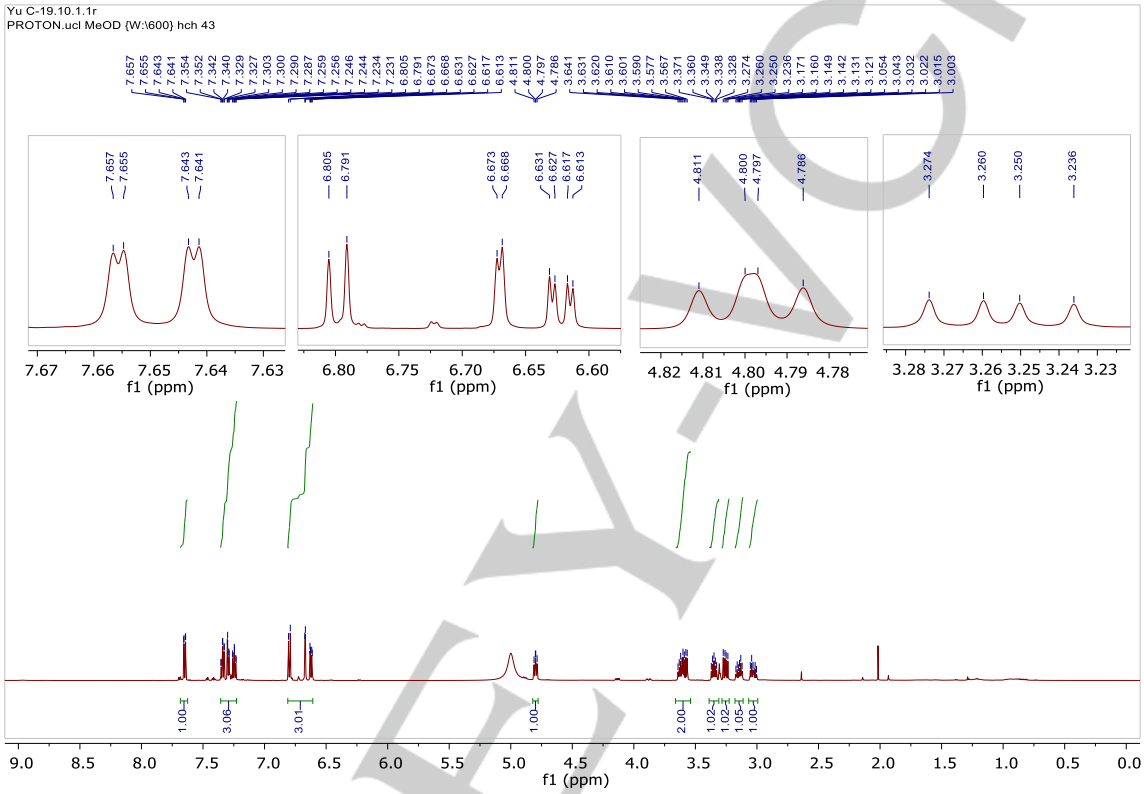
^{13}C NMR (151 MHz; CD_3OD)



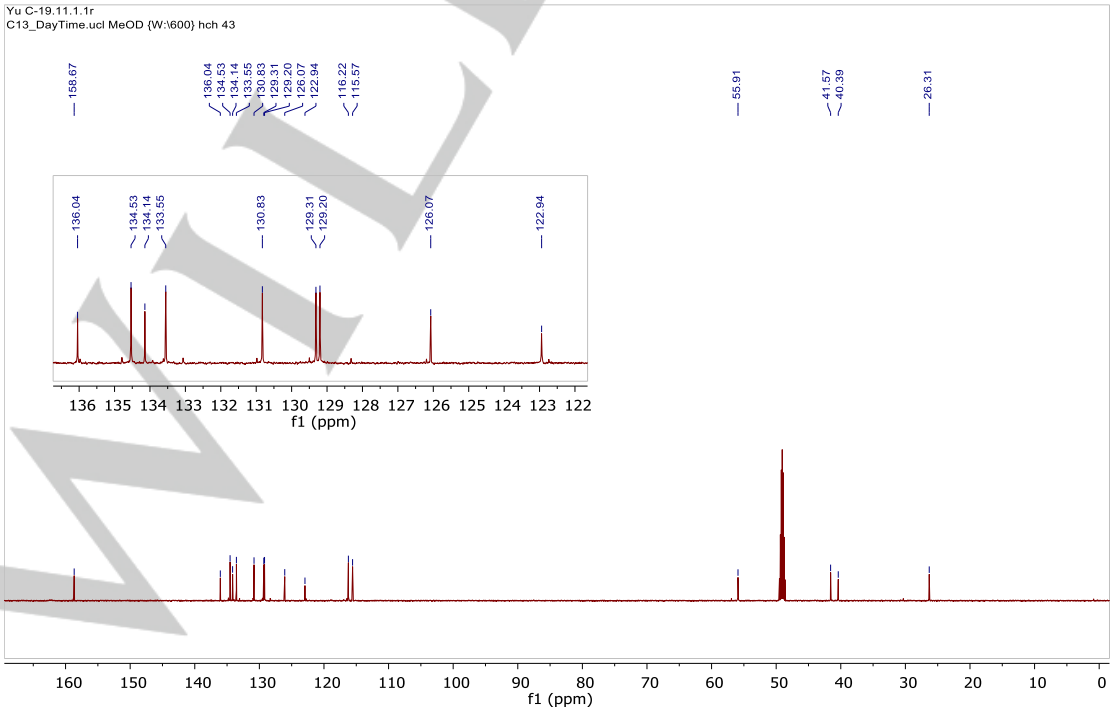
Entry 5: (*S*)-1-(4-Bromobenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**25**



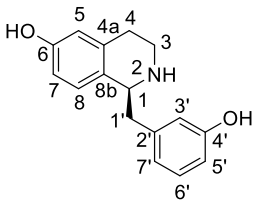
^{1}H NMR (600 MHz; CD_3OD)



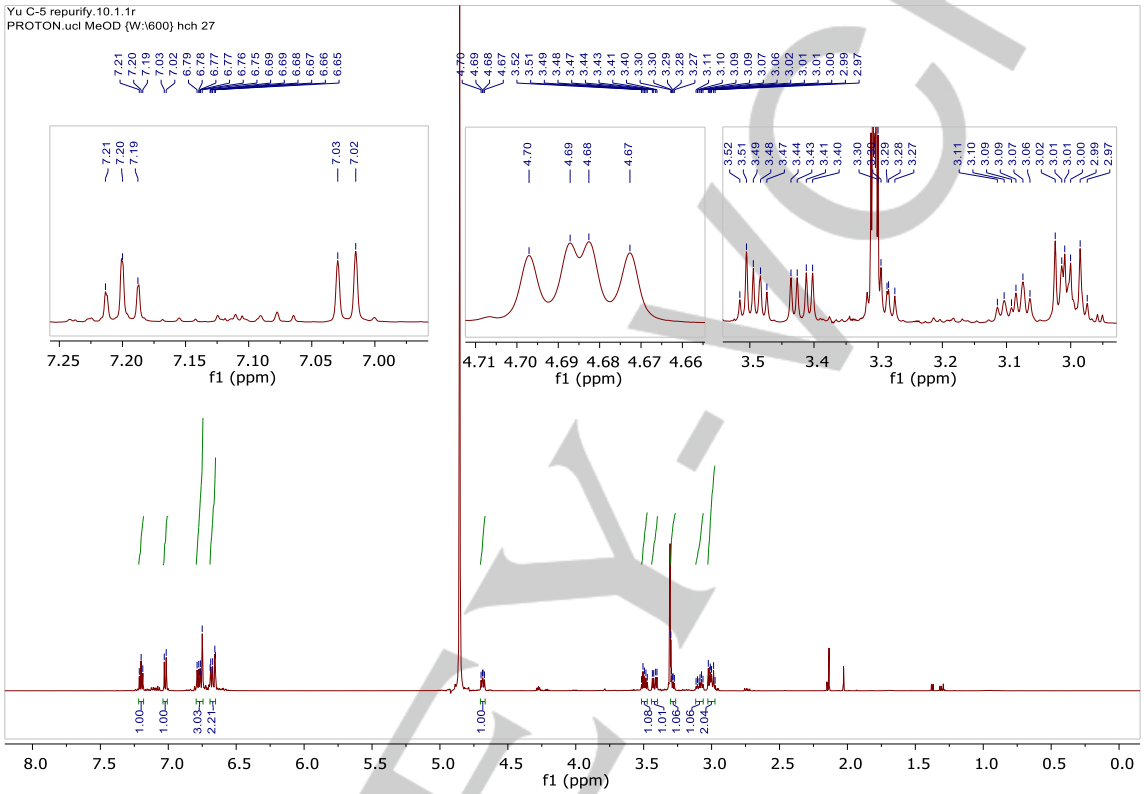
^{13}C NMR (151 MHz; CD_3OD)



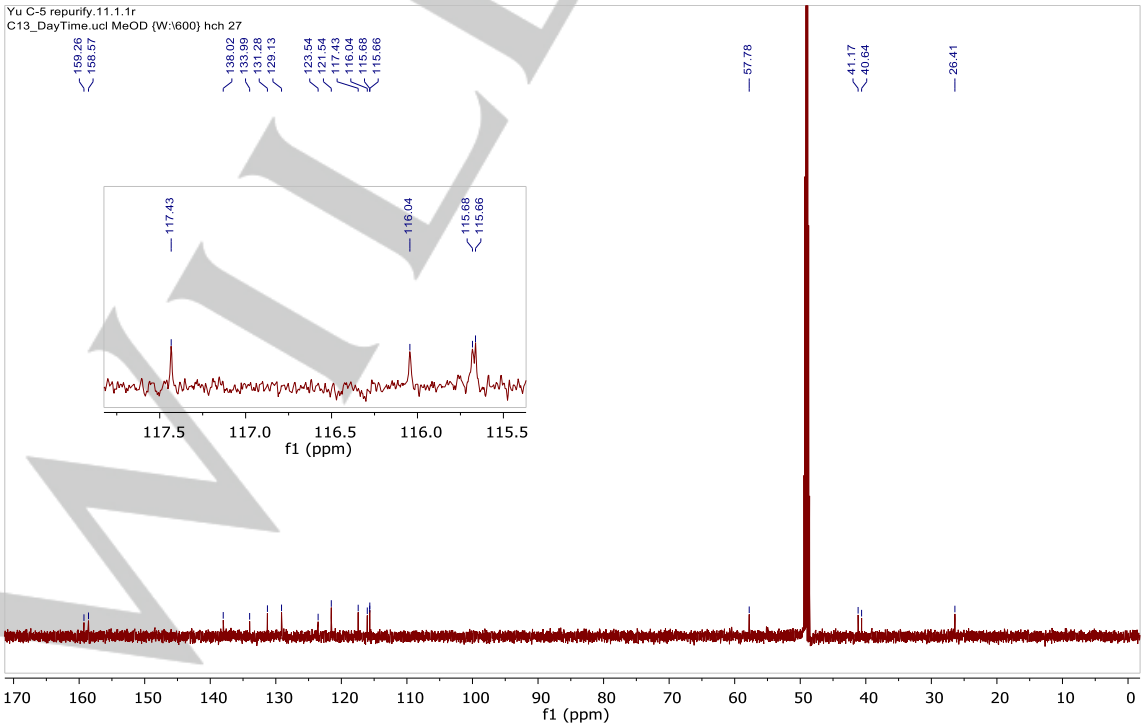
Entry 6: (*S*)-1-(3-Hydroxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol (*S*)-**27**[2]



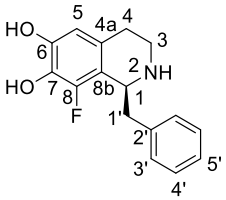
^{1}H NMR (600 MHz; CD_3OD)



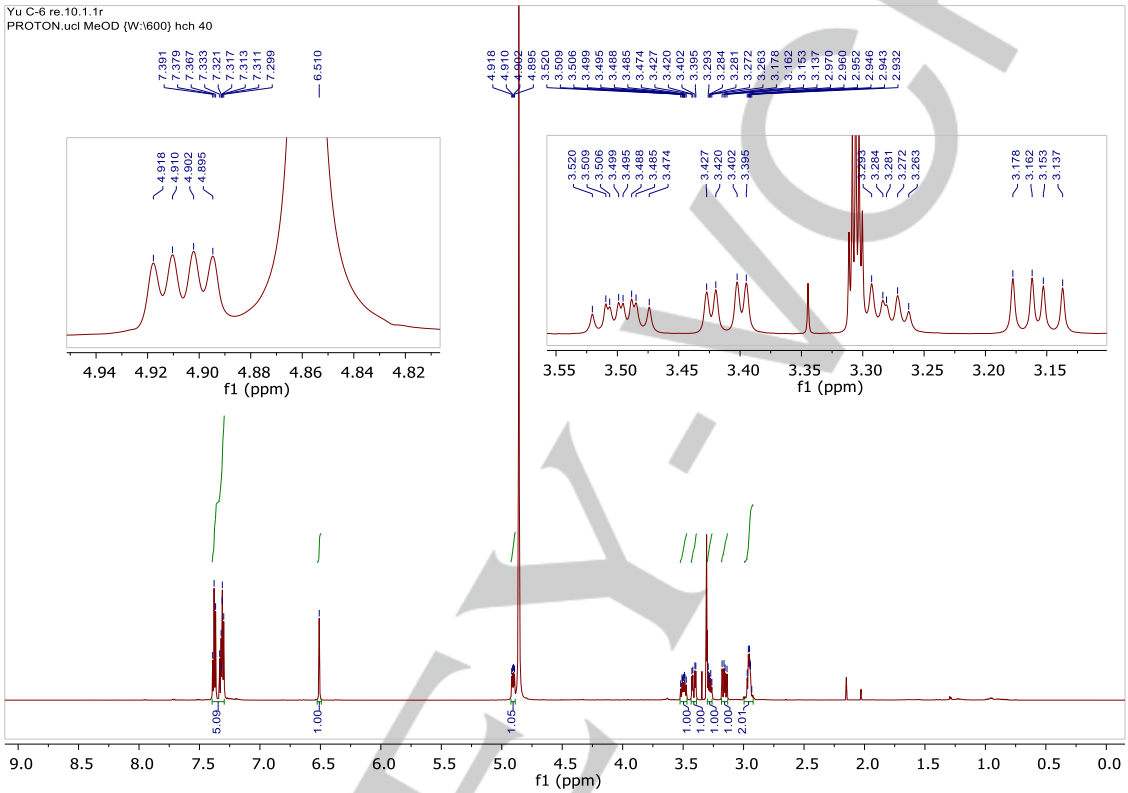
^{13}C NMR (151 MHz; CD_3OD)



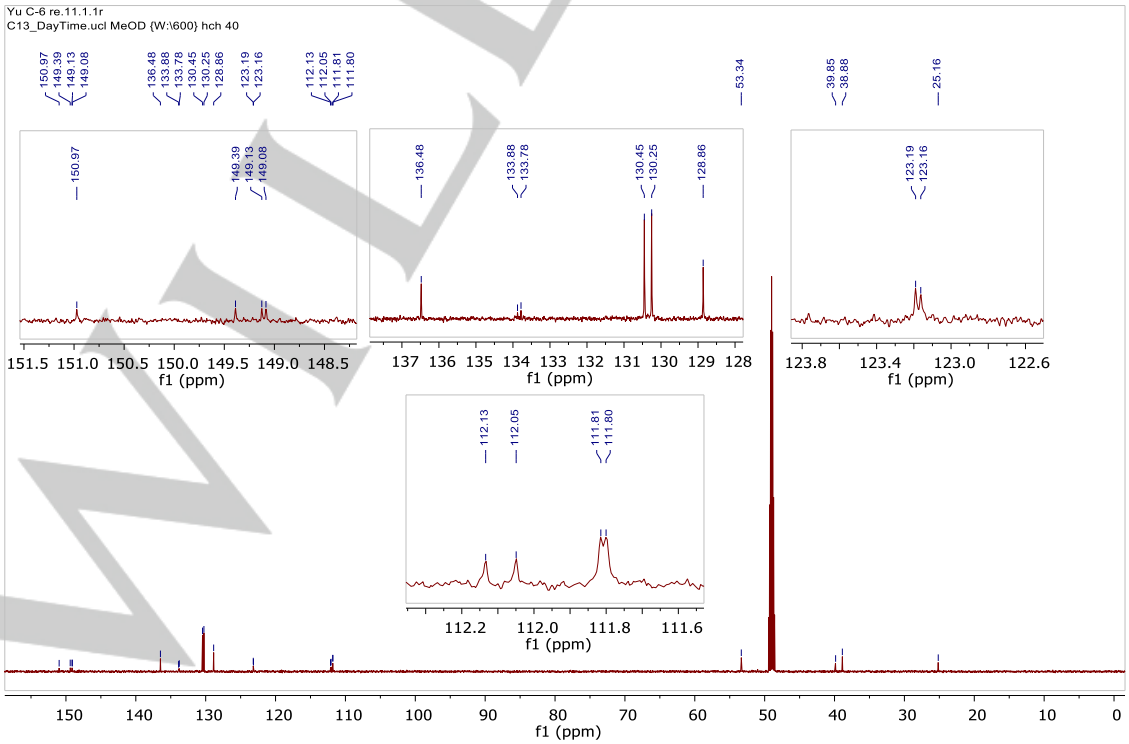
Entry 7: (*S*)-1-Benzyl-8-fluoro-1,2,3,4-tetrahydroisoquinoline-6,7-diol (*S*)-**29**



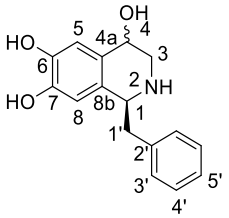
^{1}H NMR (600 MHz; CD_3OD)



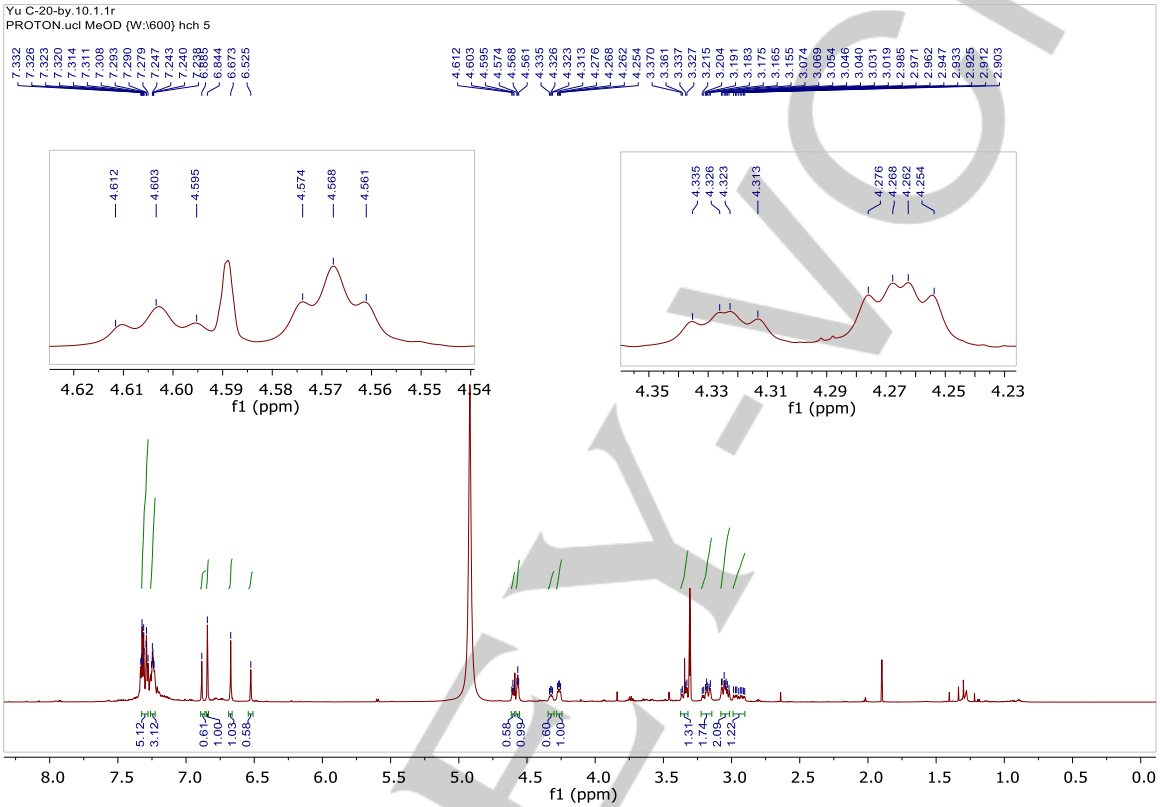
^{13}C NMR (151 MHz; CD_3OD)



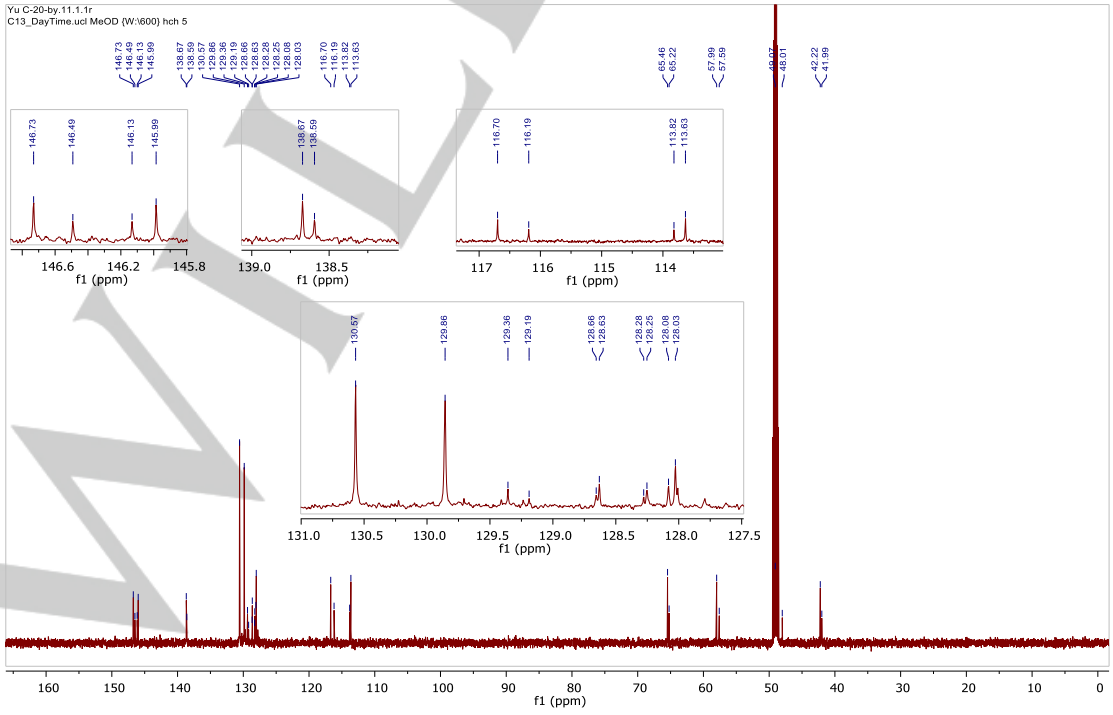
Entry 8: (1*S*, 4*RS*)-1-Benzyl-1,2,3,4-tetrahydroisoquinoline-4,6,7-triol (1*S*, 4*RS*)-**32**



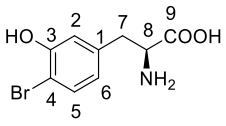
^{1}H NMR (600 MHz; CD_3OD)



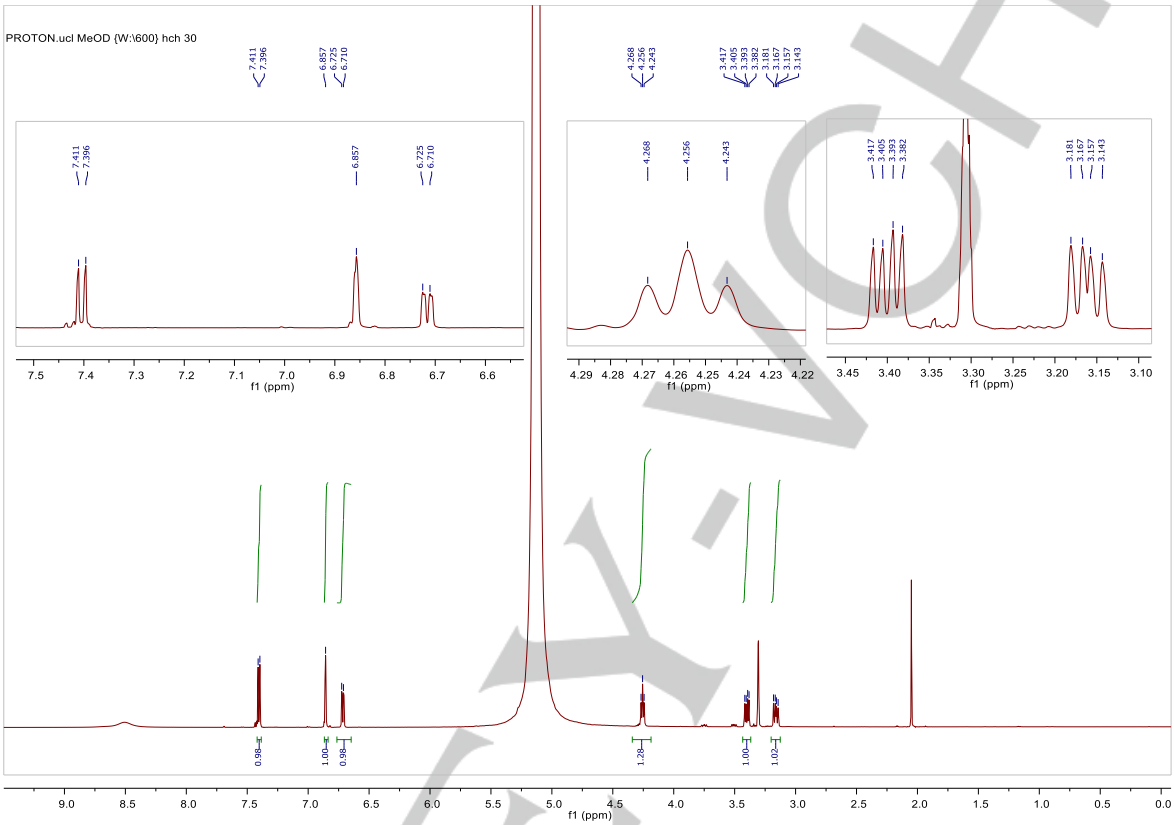
^{13}C NMR (151 MHz; CD_3OD)



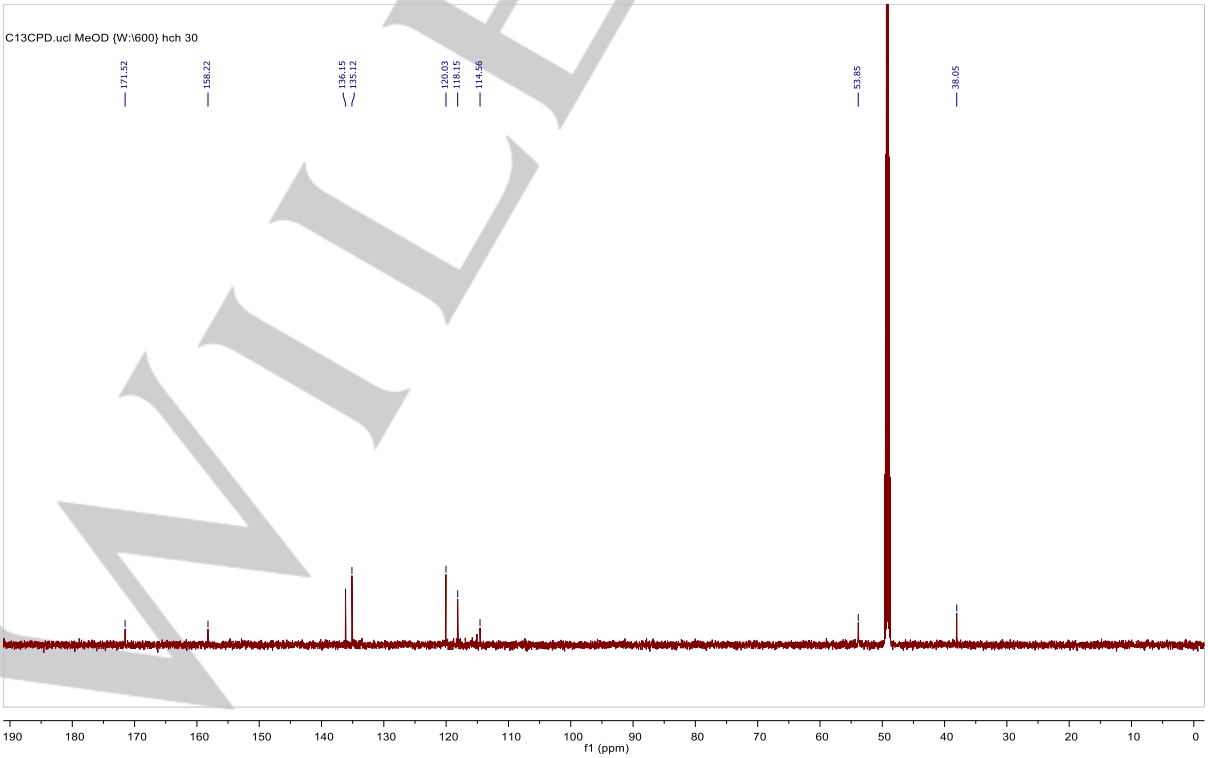
2-Amino-3-(4-bromo-3-hydroxyphenyl) propanoic acid **13**[6]



^{1}H NMR (600 MHz; D_2O and 20% CD_3OD)



^{13}C NMR (151 MHz; D_2O and 20% CD_3OD)



Reference

[1] F. Sievers, D. G. Higgins, *Methods Mol. Biol.* 2014, **1079**, 105-116.

[2] B. R. Lichman, E. D. Lamming, T. Pesnot, J. M. Smith, H. C. Hailes, J. M. Ward, *Green Chem.* **2015***, 17,* 852-855.

[3] T. Pesnot, M. C. Gershater, J. M. Ward, H. C. Hailes, *Chem. Commun.* **2010**, *47*, 3242-3244.

[4] S. Teitel, J. O'Brien, A. Brossi, *J. Med. Chem.*, **1972**, *15*, 845-846.

[5] T. Pesnot, M. C. Gershater, J. M. Ward, H. C. Haile**s**, *Adv. Synth. Catal.,* **2012***, 354,* 2997-3008.

[6] R. S. Philips, S. Busby, L. Edenfield, K. Wickware, *Amino Acids*, **2013**, *44*, 529-532.