Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting Information

Contents:

Supplementary Data

Transaminase screen

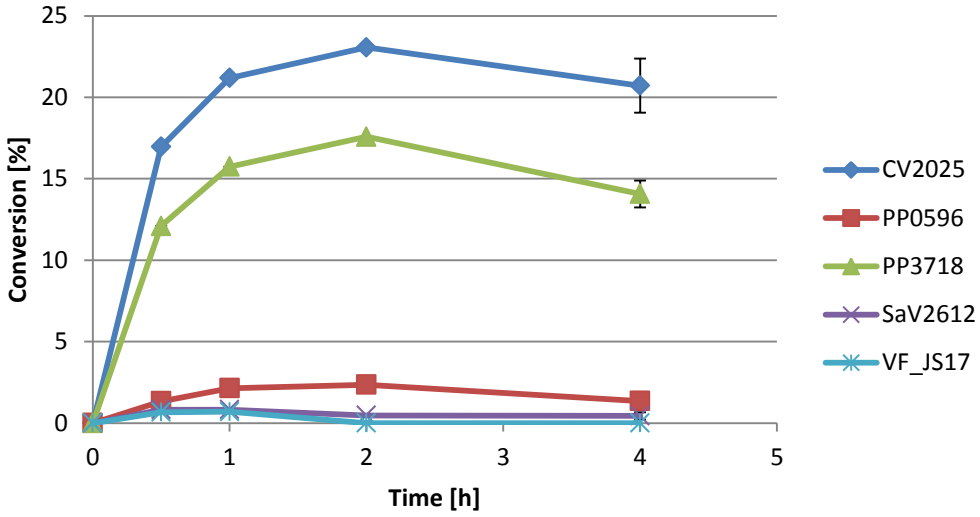

Figure S1. Progress curves charting the accumulation of *rac*-**1a** over time with different TAms. The final measurement at 4 hours was performed in triplicate. Loss of product between 2 and 4 hours is likely to be due to the instability and oxidation of the product. Only TAms showing activity towards dopamine are represented here. Conditions: 50 mM **2a**, 25 mM pyruvate, 1 mM PLP, 10% $v.v^{-1}$ TAm, 37 °C, 4 h.

Supplementary Methods

General experimental and analytic methods

Chemicals: All reagents were obtained from commercial sources and used as received unless otherwise stated. TLC was performed on Kieselgel 60 F254 precoated plastic plates and compounds visualised by exposure to UV light, potassium permanganate, phosphomolybdic acid (PMA) or ninhydrin. Flash column chromatography was carried out using silica gel (particle size 40-63 µm).

NMR: ^{1}H and ^{13}C NMR spectra were recorded at 298 K at the field indicated using Bruker AMX 300, AMX 400, Avance 500 and Avance 600 machines. Coupling constants were measured in Hertz (Hz) and referenced to the deuterated solvent used. Infrared spectra were recorded on Perkin Elmer Spectrum 100 FTIR spectrometer.

Analytical HPLC: Methods were performed with a HPLC system consisting of a LC Packing FAMOS Autosampler, a Dionex P680 HPLC Pump, a Dionex TCC-100 Column oven and a Dionex UVD170U Ultraviolet detector.

Method 1, achiral: Analytic reverse phase analysis method was used for achiral quantitative analyses. Separation was achieved with a HiChrom ACE C18-5 (150 × 4.6 mm) column and a 1 $mL.min^{-1}$ gradient of H_2O (0.1% TFA) and acetonitrile 90:10 to 30:70 at 30 °C. Injection volumes were 20 µL. Product was detected via UV absorbance at 280 nm. Product retention times and concentrations were determined by chemically verified standards (see below).

Method 2, chiral: This method was used for chiral analyses of crude products **1** and **4**. Chiral separation was achieved with a Supelco Astec Chirobiotic T column and an isocratic mobile phase 20 mM NH_4OAc pH 4:MeOH (70:30) mobile phase at 0.2 $mL.min^{-1}$ and 30 °C. Injection volumes were 5 µL. Compounds were detected by UV absorbance at 230 nm. Product retention times and concentrations were determined by chemically verified standards.

Method 3, chiral: This method was used for chiral analyses of crude product **1b**. Chiral separation was achieved with a Supelco Astec Chirobiotic T2 column and an isocratic MeOH (0.1% TFA, 0.2 % TEA) mobile phase at 1 $mL.min^{-1}$ and 30 °C. Injection volumes were 5 µL. Compounds were detected by UV absorbance at 230 nm. Product retention times and concentrations were determined by chemically verified standards.

Prep HPLC: Preparative HPLC was performed on a Varian Prostar instrument equipped with an autosampler and a UV-visible detector. Elutions were monitored at 280 nm.

Method 4: DiscoveryBIO wide Pore C18-10 Supelco column (25 × 2.12 cm), gradient: 5% to 40% of acetonitrile/water (0.1% TFA).

Method 5: Ascentis C18 150 x 21.2 mm, 5 µm. Gradient 5 -20 % of acetonitrile/water (0.1% TFA).

Transaminase preparation

Transaminase genes were amplified by PCR from genomic DNA, cloned into pET29a plasmids and expressed from BL21 (DE3) cells. Multiple sequence alignment constructed with Clustal omega.[3]

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SAV_4551        MTPQPNPQVG-----AAVKAADRAHVFH---SWSAQELIDPLAVAGAEGSYFWDYDGRRY       52
PP0596          ---MNMPETGPA---GIASQLKLDAHWMPYTANRNF-QRDPRLIVAAEGNYLVDDHGRKI       53
CV_2025         MQKQRTT--------SQWRELDAAHHLHPFTDTASLNQAGARVMTRGEGVYLWDSEGNKI       52
VF_JS17         ---MNKP--------QSWEARAETYSLYGFTDMPSLHQRGTVVVTHGEGPYIVDVNGRRY       49
BSU09260        MEMMGMEN---IQQNQGLKQKDEQFVWH---AMKGAHQADSLIAQKAEGAWVTDTDGRRY       54
PP3718          ---MATPSKAFAIAHDPLVEADKAHYMHGYHVFDEHREQGALNIVAGEGAYIRDTHGNRF       57
SAV_2612        ---MGNP---IAVSKDL-SRTAYDHLWMHFTRMSSYENAPVPTIVRGEGTYIYDDKGKRY       53
Dgeo_1416       ---MTGTKTK----ASKWLDAE-------LRYDSGVYNKHQVVMVRGQGATVWDETGRAY       46
KPN_00255       ---MNSNKAM----MA---RRS-------DAVPRGVGQIHPIFAERAENCRVWDVEGREY       43
                                                          .:   . *  *.

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PP0596          FDALSGLWTCGAGHTRKEIADAVTRQLSTLDYSPAF-QFGHPLSFQLAEKIAELVPGN-L       111
CV_2025         IDGMAGLWCVNVGYGRKDFAEAARRQMEELPFYNTFFKTTHPAVVELSSLLAEVTPAG-F       111
VF_JS17         LDANSGLWNMVAGFDHKGLIDAAKAQYERFPGYHAFFGRMSDQTVMLSEKLVEVSPFD-S       108
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PP3718          LDAVGGMWCTNIGLGREEMALAIVDQVRQLAYSNPFSDMANDVAIELCQKLAQLAPGD-L       116
SAV_2612        LDGLSGLFVVQAGHGRTELAETAFKQAQELAFFPVW-SYAHPKAVELAERLANYAPGD-L       111
Dgeo_1416       IDCVAGYGVANIGHCHPDVVKAIQEQAARLIVMPQT--LPNDKRAEFLTELVGVLPQG-L       103
KPN_00255       LDFAGGIAVLNTGHLHPQVVAAVEDQLKKLSHTCFQ-VLAYEPYLALCEKMNQKVPGDFA       102
                :*  .*      *  :  .  :   *   :                    :

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PP0596          NHVFYTNSGSECADTALKMVRAYWRLKGQATKTKIIGRARGYHGVNIAGTSLGGVNGNRK       171
CV_2025         DRVFYTNSGSESVDTMIRMVRRYWDVQGKPEKKTLIGRWNGYHGSTIGGASLGGMKYMHE       171
VF_JS17         GRVFYTNSGSEANDTMVKMLWFLHAAEGKPQKRKILTRWNAYHGVTAVSASMTGKPYNSV       168
BSU09260        -VIFFSNSGSEANETAFKIARQYHLQNGDHSRYKFISRYRAYHGNTLGALSATGQAQRKY       170
PP3718          NHVFLTTGGSTAVDTAYRLIQYYQNCRGKPHKKHIIARYNAYHGSTTLTMSIGNKAADRV       176
SAV_2612        NKVFFTTGGGEAVETAWKLAKQYFKLQGKPTKYKVISRAVAYHGTPQGALSITGLPALKA       171
Dgeo_1416       ERVFLCNSGTEAMEAAKKFAITA------TGRSRFVSMKRGFSGRSLGALAFTWE-----       152
KPN_00255       KKTLLVTTGSEAVENAVKIARAA------TGRSGAIAFTGAYHGRTHYTLSLTGKVNPYS       156
                   :  . *  . :   ::           :   :    .: *

SAV_4551        --SDSASAGVVHFWAPYLYRSRFYAETEQQECER-ALEHLET-TIAFEGPGTIAAIVLET       219
PP0596          -MFGQLLDV-DHLPHTVLPVNAFSKGLPEEGGIA-LADEMLK-LIELHDASNIAAVIVEP       227
CV_2025         -QGDLPIPGMAHIEQPWWYKHGKDM-TPDEFGVV-AARWLEE-KILEIGADKVAAFVGEP       227
VF_JS17         --FGLPLPGFVHLTCPHYWRYGEEGETEEQFVAR-LARELEE-TIQREGADTIAGFFAEP       224
BSU09260        -KYEPLSQGFLHAAPPDIYRNPDDADTLES------ANEIDR-IMTWELSETIAGVIMEP       222
PP3718          PEFDYHHDLIHHVSNPNPYRAPDDMDEA-EFLDF-LVAEFED-KILSLGADNVAAFFAEP       233
SAV_2612        P-FEPLVPGAHKVPNTNIYRAPLFGDDPEAFGRW-AADQIEQ-QILFEGPETVAAVFLEP       228
Dgeo_1416       ----------------PKYREPFGEAVDNKHVDFVTYGNIDE--LRAAVTDQTAAVILEP       194
KPN_00255       -------AGMG-LMPGHVYRALYPCALHGVSDD-EAIASIHRIFKNDAAPEDIAAIIIEP       207
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PP0596          LAGSAGVLPPPKGYLKRLREICTQHNILLIFDEVITGFGRMGAMTGSE-AFGVTPDLMCI       286
CV_2025         IQGAGGVIVPPATYWPEIERICRKYDVLLVADEVICGFGRTGEWFGHQ-HFGFQPDLFTA       286
VF_JS17         VMGAGGVIPPAKGYFQAILPILRKYDIPVISDEVICGFGRTGNTWGCV-TYDFTPDAIIS       283
BSU09260        IITGGGILMPPDGYMKKVEDICRRHGALLICDEVICGFGRTGEPFGFM-HYGVKPDIITM       281
PP3718          IMGSGGVIIPPEGYFQRMWQLCQTYDILFVADEVVTSFGRLGTFFASEELFGVTPDIITT       293
SAV_2612        VQNAGGCFPPPPGYFQRVREICDQYDVLLVSDEVICAFGRLGTMFACD-KFGYVPDMITC       287
Dgeo_1416       VQGEGGVRPVTPEFIRAAREITREKGALLILDEIQTGFCRTGKMFAAE-HFGVVPDGMTL       253
KPN_00255       VQGEGGFYAASPAFMQRLRALCDEHGIMLIADEVQSGAGRTGTLFAME-QMGVAADITTF       266
                :   .*       :      :       .: **:  .  * *   .         *

SAV_4551        AKGVNSGYVPLGGVAISGKIAETFG-------KRAYPGGLTYSGHPLACAAAVATINVMA       331
PP0596          AKQVTNGAIPMGAVIASSEIYQTFMNQPTPEYAVEFPHGYTYSAHPVACAAGLAALDLLQ       346
CV_2025         AKGLSSGYLPIGAVFVGKRVAE----GL--IAGGDFNHGFTYSGHPVCAAVAHANVAALR       340
VF_JS17         SKNLTAGFFPMGAVILGPELSKRLETAI--EAIEEFPHGFTASGHPVGCAIALKAIDVVM       341
BSU09260        AKGITSAYLPLSATAVKRDIFEAYQ-GE--APYDRFRHVNTFGGSPAACALALKNLQIME       338
PP3718          AKGLTSAYLPLGACIFSERIWQVIA-EP--GKGRCFTHGFTYSGHPVCCTAALKNIEIIE       350
SAV_2612        AKGMTSGYSPIGACIVSDRIAEPFY-K----GDNTFLHGYTFGGHPVSAAVGVANLDLFE       342
Dgeo_1416       AKAMAGG-VPVGAFAMTAEVADRMPAG---------GHGTTFGGNPLAMAAGIAAIRAMK       303
KPN_00255       AKSIAGG-FPLAGVTGRAEVMDAIAPG---------GLGGTYAGNPIACAAALAVLQIFE       316
                :* :  .  *:..     : .               * .. *   : .   :  .
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SAV_4551        EEGVVENAANLGARVIEPGLRELAERHPSVGEVRGVGMFWALELVKDRETREPLVPYNAA        391
PP0596          KENLVQSAAEL-APHFEKLLHG-VKGTKNIVDIRNYGLAGAIQIAARDGDAIVRPY----        400
CV_2025         DEGIVQRVKDDIGPYMQKRWRETFSRFEHVDDVRGVGMVQAFTLVKNKAKRELFPDFGEI        400
VF_JS17         NEGLAENVRRL-APRFEERLKH-IAERPNIGEYRGIGFMWALEAVKDKASKTPFDGNLSV        399
BSU09260        DEQLIQRSRDL-GAKLLGELQA-LREHPAVGDVRGKGLLIGIELVKDKLTKEPADAAKVN        396
PP3718          REQLLDHVNDV-GSYLEQRLQS-LRDLPLVGDVRCMKLMACVEFVANKASKALFADEVNI        408
SAV_2612        REGLNQHVLDN-ESAFLTTLQK-LHDLPIVGDVRGNGFFYGIELVKDKATKETFTDEESE        400
Dgeo_1416       NEKMAEQAREK-GAYFMERLRAI--RSPKIREVRGLGLMIGVELKEKSAPY---------        351
KPN_00255       QENLLEKANQL-GDTLRQGLLAIAEDHPEIGDVRGLGAMIAIELFEEGDHSRPNA-----        370
                 * : :         :             : : *       .

SAV_4551        GEA----NAPMAAFGAAAKANGLWPFINMNRTHVVPPCNVTEAEAKEGLAALDAALSVAD        447
PP0596          -----EAAMKLWKAGFYVRFGGDT-------LQFGPTFNTKPQELDRLFDAVGETLNLID        448
CV_2025         GTL---CRDIFFRNNLIMRACGDH-------IVSAPPLVMTRAEVDEMLAVAERCLEEFE        450
VF_JS17         SER---IANTCTDLGLICRPLGQS-------VVLCPPFILTEAQMDEMFDKLEKALDKVF        449
BSU09260        Q-----VVAACKEKGLIIGKNGDTVAGYNNVIQLAPPFCLTEEDLSFIVKTVKESFQTI-        450
PP3718          GER---IHSKAQEKGLLVRP----I---MHLNVMSPPLIITHAQVDEIVETLRQCIIETA        458
SAV_2612        RVLYGFVSKKLFEYGLYCRA----DDRGDPVIQLSPPLISNQSTFDEIESIIRQVLTEAW        456
Dgeo_1416       ------IAALEHEEGVLTLAATP------LVVRFLPPLTISREQIDQVVAAFERVLERVN        399
KPN_00255       -RLTADIVARARDKGLILLSCGPY----YNVLRILVPLTIEEAQIEQGLKIIADCFSEAK        425
                                                              .         :

SAV_4551        EYTV------------      451
PP0596          ----------------      448
CV_2025         QTLKARGLA-------      459
VF_JS17         AEVA------------      453
BSU09260        ----------------      450
PP3718          RELTALGLYQGR----      470
SAV_2612        TKL-------------      459
Dgeo_1416       PRAIPNQELREDKQTE      415
KPN_00255       QA--------------      427
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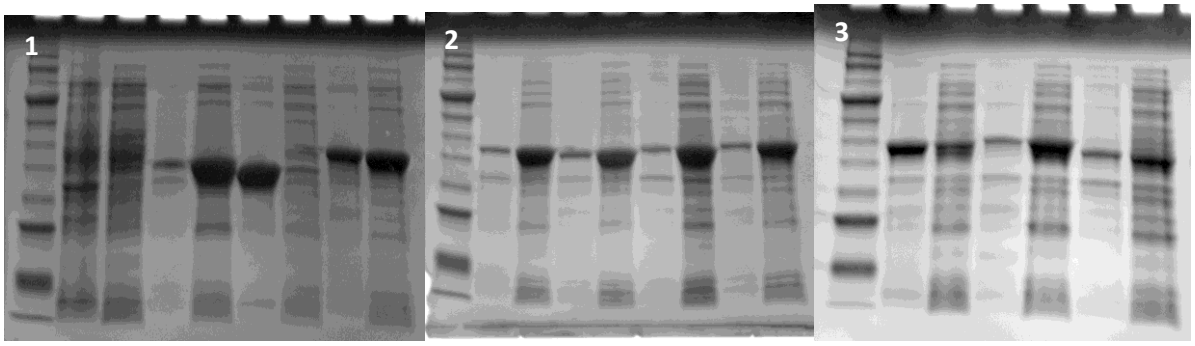
Transaminase expression: Selected TAm glycerol stocks (pET29a, BL21) from the Ward group library were plated out agar plates with kanamycin (100 $\mu g.mL^{-1}$). Single colonies from these plates were used to inoculate 5 mL 2xYT media and these were grown for 16 hours at 37 °C with 250 rpm orbital shaking. 300 µL of these overnight cultures were used to inoculate 75 mL of 2xYT media. These were incubated (37 °C, 250 rpm) until OD_{600} = 0.8. Cultures were then induced with IPTG (500 µM) and incubated at 30 °C for 8 hours. The cells were then harvested by centrifugation (10,000 × g, 10 mins) and the cell pellets stored at -80 °C.

Transaminase lysate preparation: TAm cell pellets were resuspended in lysis buffer (10% culture volume, 0.1 M potassium phosphate, 1 mM PLP, pH 7.5) and homogenised by sonication (10 s intervals, 5 cycles). The insoluble portion of the lysate was pelleted by centrifugation (10,000 × g, 30 mins). The supernatant was flash frozen in liquid nitrogen and stored at -80 °C.



Supplementary Figure S1. Gel 1, (L to R): Ladder, empty vector pellet (P), empty vector supernatant (SN), BSU_09260 (1) P, BSU_09260 (1) SN, BSU_09260 (2) P, BSU_09260 (2) SN, CV_2025 P, CV_2025 SN.
Gel 2: Ladder, Dgeo_1416 P, Dgeo_1416 SN, KPN_00255 SN, PP_0596 P, PP_0596 SN, PP_3718 P, PP_3718 SN.
Gel3: Ladder, SaV_2612 P, SaV_2612 SN, SaV_4551 P, SaV_4551 SN, VF_JS17 P and VF_JS17 SN.

CV2025 preparation for one-pot two-enzyme cascade: CV2025 was expressed in 2xYT using the general TAm method described above. The CV2025 pellets were resuspended in lysis buffer (50 mM HEPES, 10 mM PLP, pH 7.5) and homogenised by sonication (10 s intervals, 5 cycles). The insoluble portion of the lysate was pelleted by centrifugation (10,000 × g, 30 mins). The supernatant was flash frozen in liquid nitrogen and stored at -80 °C.

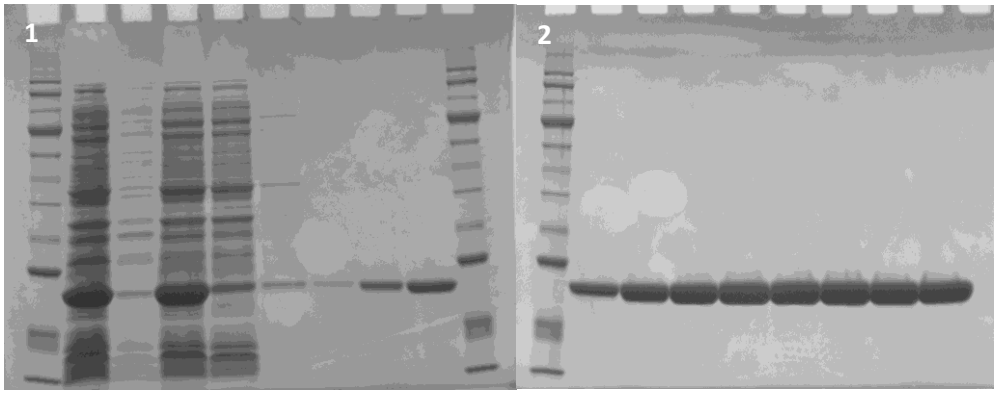
NCS Preparation

The Δ29*Tf*NCS DNA was codon optimised for recombinant expression in *E. coli* and cloned into a pJ411 vector by DNA2.0.[4] The protein was expressed in BL21 (DE3) cells.

DNA Sequence:
GTTTAACTTTTAGGAGGTAAAACAT**ATG**TTGCATCACCAGGGTATCATCAATCAAGTTAG
CACCGTCACGAAAGTAATTCATCACGAGCTGGAAGTTGCGGCATCCGCTGACGACATTTG
GACCGTGTACAGCTGGCCGGGTCTGGCGAAGCACTTGCCGGATCTGCTGCCTGGCGCGTT
CGAAAAACTGGAGATTATCGGCGATGGCGGTGTTGGTACGATTCTGGACATGACCTTTGT
CCCGGGTGAATTCCCGCACGAGTATAAAGAGAAATTCATCCTGGTTGATAACGAACATC
GTCTGAAGAAGGTGCAGATGATCGAAGGCGGCTATCTGGACCTGGGTGTGACGTATTAC
ATGGACACGATTCACGTTGTGCCGACCGGTAAAGACAGCTGCGTCATCAAGAGCAGCAC
TGAGTACCACGTCAAGCCGGAGTTTGTGAAGATTGTTGAGCCGCTGATCACCACCGGTCC
ACTGGCAGCCATGGCAGATGCCATTAGCAAGTTGGTCCTGGAACATAAATCTAAAAGCA
ACTCCGATGAAATTGAGGCGGCGATCATCACCGTGCTGGAGCATCACCACCACCATCAC
TGATAAAAGCTTCCCC

Expression of NCS: Plasmids containing a codon optimised Δ29*Tf*NCS were purchased from DNA2.0 and transformed into E. coli BL21(DE3) cells by a standard heat-shock protocol. The transformed cells were stored as glycerol stocks at -80 °C.An aliquot from a frozen glycerol stock of plasmid containing *E. coli* BL21(DE3) was inoculated into 20 mL of TB medium (containing 50 $\mu g.mL^{-1}$ kanamycin). Starter cultures were incubated overnight at 37 °C, shaking at 250 rpm. A 4 mL sample of overnight starter cultures was added to 100 mL of TB medium. The expression cultures were incubated for 2 hours at 37 °C, followed by 1 hour at 25 °C, whilst shaking at 250 rpm. Expression was induced by the addition of 100 µL of 500 mM IPTG. Cultures were incubated for a further 3 hours at 25 °C prior to harvesting, whilst shaking at 250 rpm. Cells were harvested by centrifugation at 10,000 × g for 10 mins at 4 °C. Supernatant was removed and the cells stored at -20 °C until purification.

Purification of NCS: Cell pellets were thawed and suspended in BugBuster (10 mL) (Novagen). The insoluble portion of the lysate was pelleted by centrifugation at 10,000 × g for 30 minutes at 4 °C. The supernatant was removed and filtered through a glass fibre prefilter and 0.2 µm cellulose acetate syringe filter. An empty and clean PD-10 column (GE) was charged with Ni-NTA (2 mL). The column was washed with distilled water (10 mL), followed by binding buffer (0.1 M HEPES, 20 mM imidazole, 100 mM NaCl, pH 7.5; 10 mL). The filtered supernatant was passed through the Ni-NTA column, and the column was then washed with binding buffer (10 mL) followed by wash buffer (0.1 M HEPES, 40 mM imidazole, 100 mM NaCl, pH 7.5; 20 mL). The bound protein was then eluted with elution buffer (0.1 M HEPES, 500 mM imidazole, 100 mM NaCl, pH 7.5; 5 mL). The eluant containing pure enzyme was buffer exchanged into 0.1 M HEPES pH 7.5, using a PD-10 column. Glycerol was added (10% v/v) and the concentration of the protein determined by absorbance at 280 nm. The protein was divided into 0.5 mL samples and frozen in liquid nitrogen. The purified protein was stored at -80 °C.



Supplementary Figure S2. Gel 1, (L to R): Ladder, lysate, pellet, supernatant, flow-through, wash 1 (20 mM imidazole), wash 2 (20 mM), wash 3 (40 mM), wash 4 (40 mM). Gel 2: 500 mM imidazole elution.

Biocatalytic cascades

Transaminase screen

Transaminases were screened for activity with dopamine and pyruvate through the formation of *rac*-**1a**. The reaction conditions: 50 mM dopamine **2a**, 25 mM pyruvate, 1 mM PLP, 10% v/v TAm lysate. The reactions (50 µL) were incubated at 37 °C with 500 rpm orbital rotation. The reactions were quenched by addition of 10 µL 1M HCl. Reactions were quenched after 30 mins, 1, 2 and 4 hours. The insoluble portions of the reactions were removed by centrifugation and the soluble fractions were analysed by analytic HPLC (method 1) and compared to chemically verified standards.

One-pot two-enzyme cascade for the synthesis of (*S*)-1a

Reactions were conducted to screen for optimum conditions. The reactions (100 µL) contained dopamine, pyruvate, purified Δ29*Tf*NCS and CV2025 lysate in varying concentrations. Conditions were: either 20 or 50 mM dopamine **2a**, (and 10 mM and 25 mM sodium pyruvate respectively), 0.1 or 0.5 mg.mL^{-1} NCS, 10, 20 or 30 % CV2025 lysate. Reactions were quenched with 20 µL 1 M HCl. Reactions were incubated at 37 °C with 500 rpm orbital rotation and were quenched after 3 hours. Each condition was performed in triplicate. The insoluble material was removed by centrifugation and the reaction were analysed by analytical and chiral HPLC (methods 1 and 2).

One-pot two-enzyme cascade of (*S*)-1b

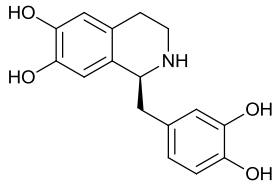
Reaction components 3-hydroxyphenethylamine **2b** (20 mM), pyruvate (10 mM), CV2025 lysate (20% v/v) and Δ29*Tf*NCS (0.5 mg.mL^{-1}) were combined (total volume 100 µL) and incubated at 37 °C with 500 rpm shaking for 3 hours. Reactions were quenched with 1M HCl (20 µL), centrifuged and then analysed by analytical and chiral HPLC (methods 1 and 3).

One-pot two-enzyme chemoenzymatic cascade for the synthesis of (*S*)-4 and 5

First, synthesis of **(*S*)-**1a was achieved with conditions determined previously: reactions (100 µL) consisting of **2a** (20 mM), pyruvate (10 mM), Δ29*Tf*NCS (0.5 mg/mL) and CV2025 lysate (20% v/v) were incubated at 37 °C with 500 rpm shaking for 3 hours. To these reactions was added 100 µL of formaldehyde/phosphate solution (40 mM formaldehyde, 1 M sodium phosphate, pH 6) and the reactions were incubated for 30 minutes at 37 °C and with 500 rpm shaking. Reactions were centrifuged to remove insoluble matter and then analysed by analytical and chiral HPLC (methods 1 and 2).

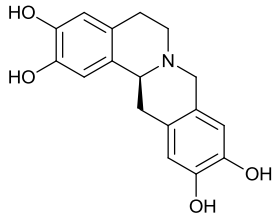
Preparatory scale enzyme cascade reactions

(*S*)-1-(3,4-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol, (*S*)-1a



Dopamine (0.5 mmole, 94.5 mg), pyruvate (0.25 mmole, 27.5 mg), CV2025 lysate (5 mL) and Δ29*Tf*NCS (12.5 mg) were combined with water to a total volume of 25 mL. The reaction was stirred at 37 °C, checking reaction progress by HPLC analysis (method 1). Once the reaction had completed (86 % conversion, 2 hours), the reaction was quenched by the addition of HCl (1M, 5 mL). Insoluble material was removed through centrifugation and ultrafiltration, and the mixture was concentrated *in vacuo*. The product was purified by preparatory HPLC (method 4) for characterisation purposes (TFA salt, 63 mg, 62% isolated yield). ^{1}H NMR and chiral HPLC confirmed the product was the expected (*S*)-**1a** (>95 % *ee*).

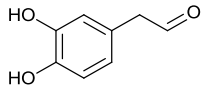
(*S*)-6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,10,11-tetraol, (*S*)-4



Dopamine (0.5 mmole, 94.5 mg), pyruvate (0.25 mmole, 27.5 mg), CV2025 lysate (5 mL) and Δ29*Tf*NCS (12.5 mg) were combined with water to a total volume of 25 mL. The reaction was stirred at 37 °C for 2 hours. Formaldehyde (40 mM in 1M phosphate pH 6, 25 mL) was added to the reaction and it was stirred at 37 °C for 30 minutes (47% conversion). Insoluble material was then removed through centrifugation and ultrafiltration, and the mixture was concentrated *in vacuo*. The product was purified by preparatory HPLC (method 4) for characterisation purposes, (TFA salt, 43 mg, 42% isolated yield). ^{1}H NMR and chiral HPLC confirmed the product was the expected (*S*)-**4** (>95 % *ee*).

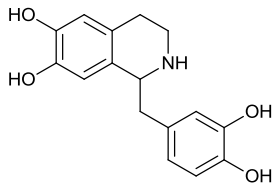
Chemical syntheses

(3,4-Dihydroxyphenyl)acetaldehyde, 3a



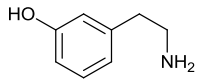
Aldehyde **3a** was prepared by the Parikh-Doering oxidation of 4-(2-hydroxyethyl)benzene-1,2-diol[5] (500 mg, 3.24 mmol). To a solution of 4-(2-hydroxyethyl)benzene-1,2-diol (1 eq.) in DMSO/CH_2Cl_2 (15 mL) was added *N*,*N*-diisopropylethylamine (2.5 eq.) followed by a solution of SO_3.pyridine (2.5 eq.) in a 1:1 mixture of DMSO/CH_2Cl_2 (10 mL), added over 30 min at -15 ºC. The mixture was stirred for 1 h at -15 ºC and quenched by the addition of ice-cold water (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and the organic layers were combined and concentrated under reduced pressure. The crude material was purified using flash silica chromatography (60% EtOAc in hexane) to give **3a** as a pale yellow oil (212 mg, 43%).[6,7] R_f 0.15 (EtOAc:hexane, 1:1); ^{1}H NMR (600 MHz; $CDCl_3$) δ = 3.59 (2H, d, *J* = 2.4 Hz, CH_2), 5.51 (1H, br s, OH), 5.76 (1H, br s, OH), 6.64 (1H, dd, *J* = 8.0 and 2.0 Hz, 6-H), 6.70 (1H, d, *J* = 2.0 Hz, 2-H), 6.85 (1H, d, *J* = 8.0 Hz, 5-H), 9.69 (1H, t, *J* 2.4 Hz, CHO); ^{13}C NMR (150 MHz; $CDCl_3$) δ = 49.9, 115.9, 116.6, 122.3, 124.2, 143.1, 144.0, 200.6; *m/z* [HRMS ES+] found $[M]^+$ 152.04679. $C_8H_8O_3$ requires 152.04680.

1-(3,4-Dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline-6,7-diol, 1a



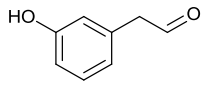
Compound **1a** was prepared by a biomimetic pictet-spengler reaction. **2a**.HCl (75 mg, 0.39 mmol) and **3a** (40 mg, 0.26 mmol) were added to 6 mL of a 1:1 mixture of acetonitrile/potassium phosphate buffer (0.1 M solution at pH 6). The resulting solution was stirred at 50 ºC for 12 h. The crude product was concentrated *in vacuo*, then purified by preparative HPLC (method 4, r_t (retention time) 11.5 min). Fractions containing the desired product were combined, concentrated and co-evaporated with methanol (3 × 20 mL) to give **1a** as a colourless oil (46 mg, 61%).[8] ^{1}H NMR (600 MHz; CD_3OD) δ = 2.86–3.02 (3H, m, 4-H_2 and NCHC*H*H), 3.23 (1H, app. quintet, *J* = 6.0 Hz, 3-*H*H), 3.30–3.36 (1H, m, NCHCH*H*), 3.44 (1H, app. quintet, *J* = 5.9 Hz, 3-H*H*), 4.54 (1H, dd, *J* = 8.8 and 5.6 Hz, 1-H), 6.61–6.65 (3H, m, 5-H, 8-H and 6'-H), 6.74 (1H, d, *J* = 1.7 Hz, 2'-H), 6.78 (1H, d, *J* = 8.0 Hz, 5'-H); ^{13}C NMR (150 MHz; CD_3OD) δ = 25.7, 40.7, 40.9, 57.9, 114.0, 116.1, 116.9, 117.4, 121.8, 123.6, 123.8, 127.7, 145.8, 146.1, 146.6, 146.8; *m/z* [HRMS ES+] found $[M+H]^+$ 288.1236. $C_{16}H_{18}NO_4$ requires 288.1233.

2-(3-Hydroxyphenyl)ethylamine hydrobromide, 2b



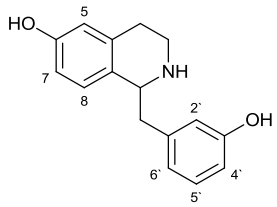
The reaction was performed under anhydrous conditions. A solution of 1 M boron tribromide in CH_2Cl_2 (10.2 mL, 10.2 mmol) was added to a stirred solution of 2-(3-methoxyphenyl)ethan-1-amine (0.700 g, 4.63 mmol) in CH_2Cl_2 (20 mL) at -78 °C under Ar. The reaction was warmed to room temperature and stirred for 24 h. The reaction was then cooled to 0 °C and quenched by addition of methanol (40 mL). The solution was stirred at room temperature for 3 h. The solution was concentrated *in vacuo* to give a brown oil. Further methanol (20 mL) was added, and solvent evaporated. This was repeated until no white fumes were observed upon addition of methanol, to give **2b** as a pale brown solid as the hydrobromide salt (0.899 g, 89%).[9] m.p. 102-104 °C; ^{1}H NMR (600 MHz; CD_3OD) δ = 2.88 (2H, t, *J* = 8.0 Hz, C*H*$_2$CH$_2$N), 3.15 (2H, t, *J* = 8.0 Hz, CH$_2$N), 6.68-6.72 (2H, m, 2-H and 4-H), 6.73 (1H, d, *J* = 7.5 Hz, 6-H), 7.16 (1H, t, *J* = 7.5 Hz, 5-H); ^{13}C NMR (150 MHz; CD_3OD) δ = 34.5, 41.9, 115.2, 116.6, 120.7, 131.0, 139.3, 159.1; *m/z* [HRMS ES+] found $[M+H]^+$ found 138.091213. $C_8H_{11}ON$ requires 138.09189.

2-(3-Hydroxyphenyl)acetaldehyde, 3b



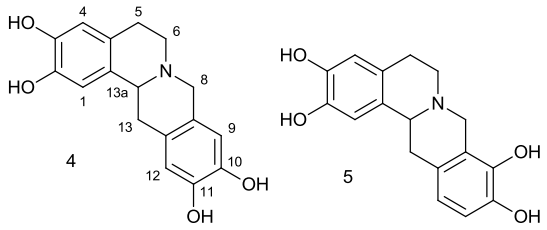
Aldehyde **3b** was prepared by Parikh-Doering oxidation of 2-(3-hydroxylphenyl)ethanol (500 mg, 3.62 mmol). To a solution of 2-(3-hydroxylphenyl)ethanol (1 eq.) in DMSO/CH_2Cl_2 (15 mL) was added *N*,*N*-diisopropylethylamine (2.5 eq.) followed by a solution of SO_3.pyridine (2.5 eq.) in a 1:1 mixture of DMSO/CH_2Cl_2 (10 mL) added over 30 min at -15 °C. The mixture was stirred for 1 h at -15 °C and quenched by the addition of ice-cold water (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL) and the organic layers were combined and concentrated under reduced pressure. The crude material was purified using silica flash chromatography (eluent 20% EtOAc in hexane). Fractions containing the desired product were combined and concentrated under reduced pressure. Purification by flash silica chromatography (15% EtOAc in hexane) yielded **3b** as a pale yellow oil (100 mg, 20%).[10] R_f 0.47 (EtOAc:hexane, 1:1); ^{1}H NMR (500 MHz; $CDCl_3$) δ = 3.64 (2H, d, *J* = 2.4 Hz, CH_2), 5.46 (1H, br s, OH), 6.69 (1H, t, *J* = 2.0 Hz, 2-H), 6.75–6.79 (2H, m, 4-H and 6-H), 7.23 (1H, t, *J* = 6.4 Hz, 5-H), 9.73 (1H, t, *J* = 2.4 Hz, CHO); ^{13}C NMR (125 MHz; $CDCl_3$) δ = 50.4, 114.6, 116.6, 122.0, 130.3, 133.4, 156.3, 199.8; *m/z* [HRMS ES+] found $[M]^+$ 136.05206. $C_8H_8O_2$ requires 136.05188.

1-(3-Hydroxybenzyl)-1,2,3,4-tetrahydroisoquinolin-6-ol, 1b



A solution of 2-(3-hydroxyphenyl)acetaldehyde **3b** (27.2 mg, 0.200 mmol) in acetonitrile (2 mL) was added to a solution of **2b** (35.0 mg, 0.160 mmol) in potassium phosphate buffer (2 mL, 0.1 M, pH 6). The solution was stirred at 50 ^{o}C for 17 h. The reaction was concentrated and purified by preparative HPLC (method 5, r_t = 30 min). Fractions containing the desired product were combined, concentrated and co-evaporated with methanol (3 x 10 mL), to give **1b** as a pale yellow oil (15.0 mg, 37%). ^{1}H NMR (CD_3OD; 600 MHz) δ = 2.96-3.03 (2H, m, 4-*H*H and NCHC*H*H) 3.05-3.12 (1H, m, 4-H*H*), 3.26-3.31 (1H, m, 3-*H*H), 3.40-3.45 (1H, m, NCHCH*H*), 3.46-3.52 (1H, m, 3-H*H*), 4.66-4.70 (1H, m, 1-H), 6.65 (1H, s, 5-H), 6.68 (1H, d, *J* = 8.5 Hz, 7-H), 6.73-6.80 (3H, m, 2`-H, 6`-H, 4`-H), 7.03 (1H, d, *J* = 8.5 Hz, 8-H), 7.12 (1H, t, *J* = 7.5 Hz, 5`-H); ^{13}C NMR (150 MHz; CD_3OD) δ = 24.9, 39.1, 39.6, 56.2, 114.1 (2 x signals), 114.5, 115.8, 119.9, 122.0, 127.5, 129.7, 132.4, 136.4, 157.0, 157.7); *m/z* [HRMS ES+] found $[M+H]^+$ 256.133461. $C_{16}H_{18}O_2N$ requires 256.13375.

Synthesis of 6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,10,11-tetraol, 4 and **6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,9,10-tetraol, 5**



Compound **1a** (30 mg, 100 µmol) and formaldehyde (15 µL, 0.20 mmol) were added to a 1:1 mixture of acetonitrile/potassium phosphate buffer (0.5 M solution at pH 6, 3 mL). The solution was stirred at 40 ºC for 0.5 h. The crude product was purified by preparative HPLC (method 4) and fractions containing the desired product were combined, concentrated and co-evaporated with methanol (3 × 20 mL). Two products were isolated 6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,10,11-tetraol **4**[11] (22.6 mg, 72%, r_t (retention time) 11.6 min) and 6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,9,10-tetraol **5**[11] (5.3 mg, 17% r_t (retention time) 12.6 min).

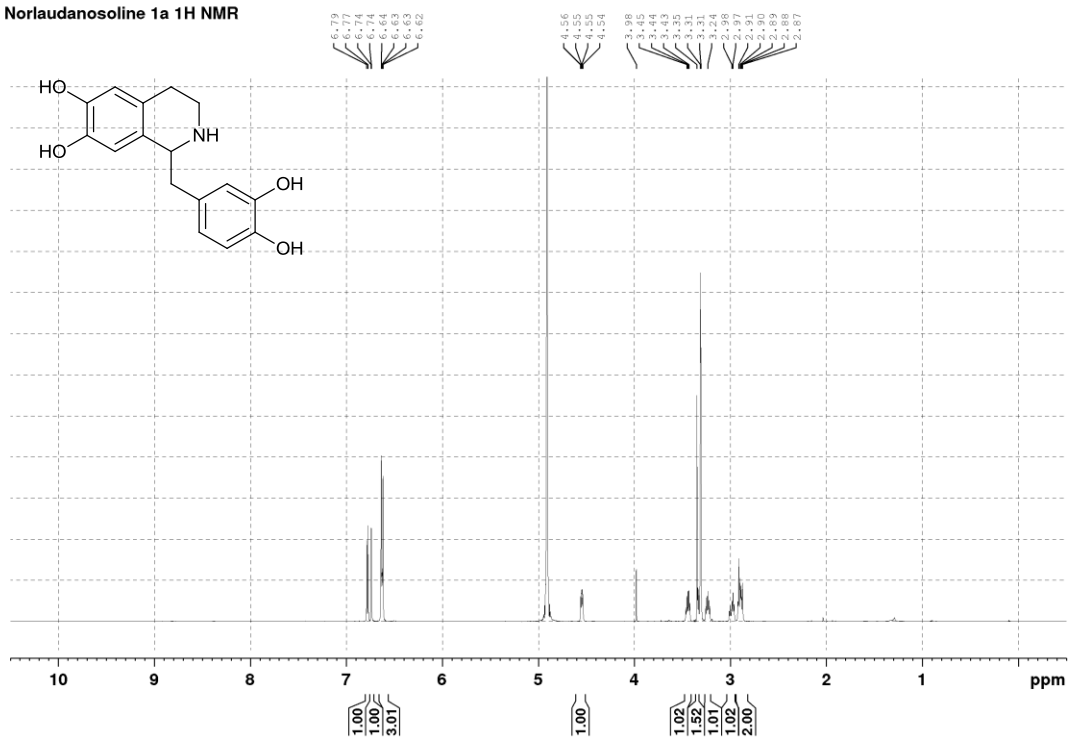
Major regioisomer **6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,10,11-tetraol (4)**: ^{1}H NMR (600 MHz; CD_3OD) δ = 2.88–3.03 (2H, m, 5-*H*H and 13-*H*H), 3.12–3.23 (1H, m, 5-H*H*), 3.48 (1H, td, *J* = 12.3 and 4.5 Hz, 6-*H*H), 3.58–3.66 (1H, m, 13-H*H*), 3.75–3.82 (1H, m, 6-H*H*), 4.38-4.50 (2H, m, 8-H_2), 4.67 (1H, dd, *J* = 11.9 and 4.6 Hz, 13a-H), 6.62 (1H, s, 9-H), 6.64 (1H, s, 4-H), 6.71 (1H, br.s, 12-H), 6.79 (1H, br.s, 1-H); ^{13}C NMR (150 MHz; CD_3OD) δ = 24.8, 32.7, 51.0, 55.1, 60.5, 111.3, 111.7, 114.4, 114.5, 117.6, 117.9, 121.6, 122.3, 144.7, 144.8, 145.4, 145.6; *m/z* [HRMS ES+] found $[M+H]^+$ 300.1228. $C_{17}H_{18}NO_4$ requires 300.1236.

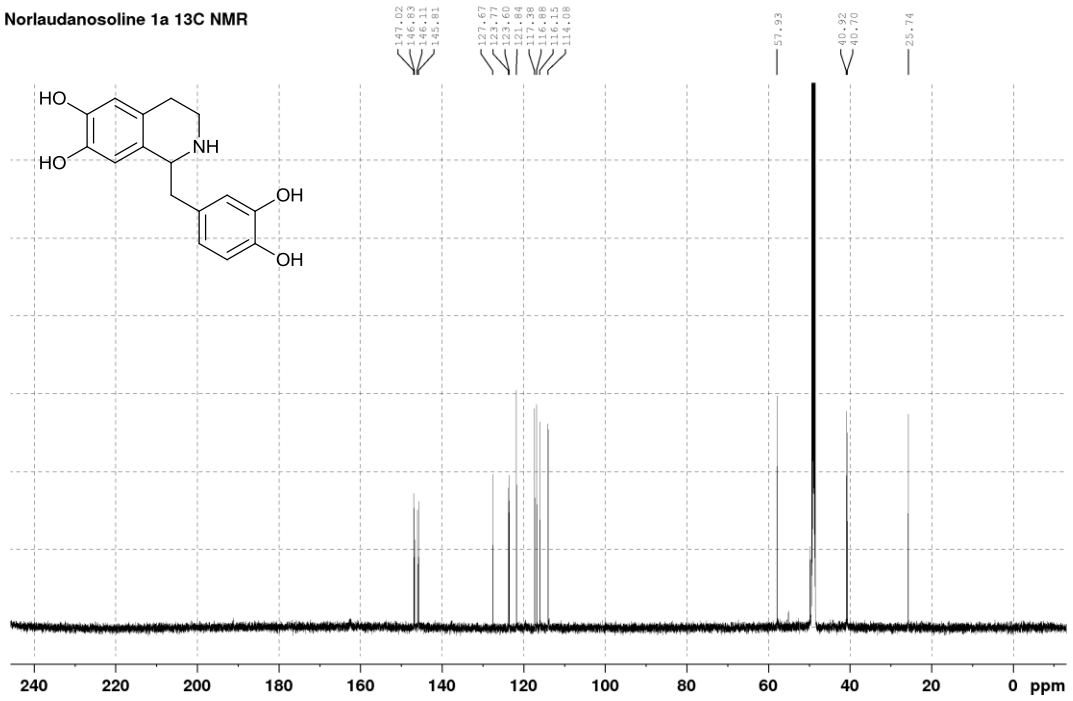
Minor regioisomer **6,8,13,13a-tetrahydro-5H-isoquinolino[3,2-a]isoquinoline-2,3,9,10-tetraol (5)**: ^{1}H NMR (600 MHz; CD_3OD) δ = 2.93 (1H, br.d, *J* = 16.8 Hz, 5-*H*H), 3.02 (1H, dd, *J* = 16.8 and 12.3 Hz, 13-*H*H), 3.16-3.25 (1H, m, 5-H*H*), 3.53 (1H, td, *J* = 12.2 and 4.7 Hz, 6-*H*H), 3.67 (1H, dd, *J* =

16.8 and 4.0 Hz, 13-H*H*), 3.88 (1H, dd, *J* = 12.2 and 4.6 Hz, 6-H*H*), 4.31 (1H, d, *J* = 15.7 Hz, 8-*H*H), 4.66 (1H, dd, *J* = 12.3 and 4.0 Hz, 13a-H), 4.73 (1H, d, *J* = 15.7 Hz, 8-H*H*), 6.65 (1H, s, 4-H), 6.67 (1H, d, *J* = 8.2 Hz, 12-H), 6.77-6.82 (2H, m, 1-H and 11-H); ^{13}C NMR (150 MHz; CD_3OD) δ = 26.5, 34.3, 52.7, 53.4, 61.7, 112.9, 115.9, 166.4, 120.3, 123.3, 123.5, 123.6, 123.8, 143.1, 144.6, 146.5, 146.9; *m/z* [HRMS ES+] found $[M+H]^+$ 300.1219. $C_{17}H_{18}NO_4$ requires 300.1236.

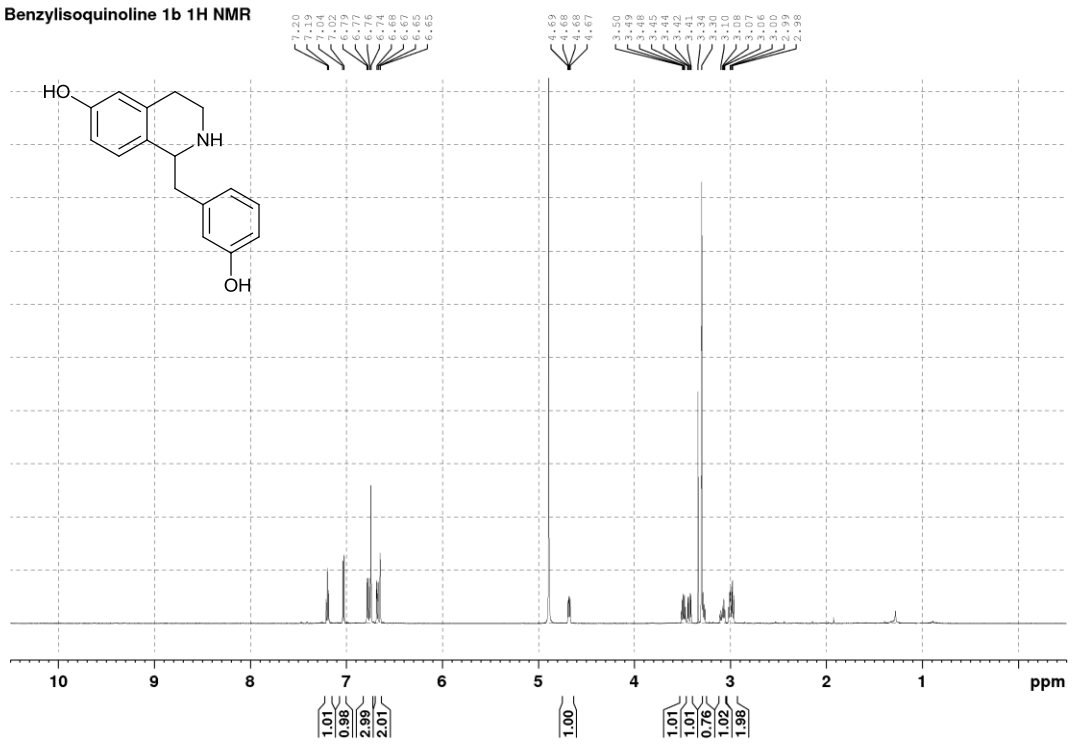
NMRs of chemical standards

1a





1b


Benzylisoquinoline 1b 1H NMR
HO
NH
OH
7.20
7.19
7.04
7.02
6.79
6.77
6.76
6.74
6.68
6.67
6.65
6.65
4.69
4.68
4.68
4.67
3.50
3.49
3.48
3.46
3.44
3.42
3.41
3.34
3.30
3.10
3.08
3.07
3.06
3.00
2.99
2.98
10
9
8
7
6
5
4
3
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1
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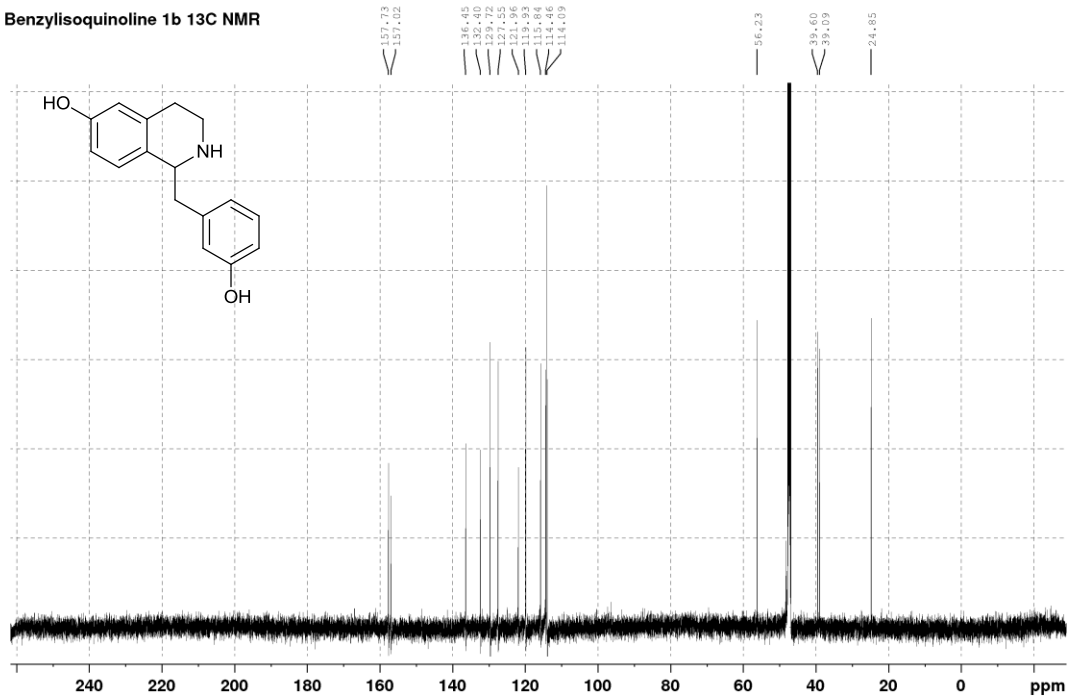
2.01
1.00
1.01
1.01
0.76
1.02
1.98


Benzylisoquinoline 1b 13C NMR
HO
NH
OH
157.73
157.02
136.45
132.40
129.72
121.55
121.06
119.93
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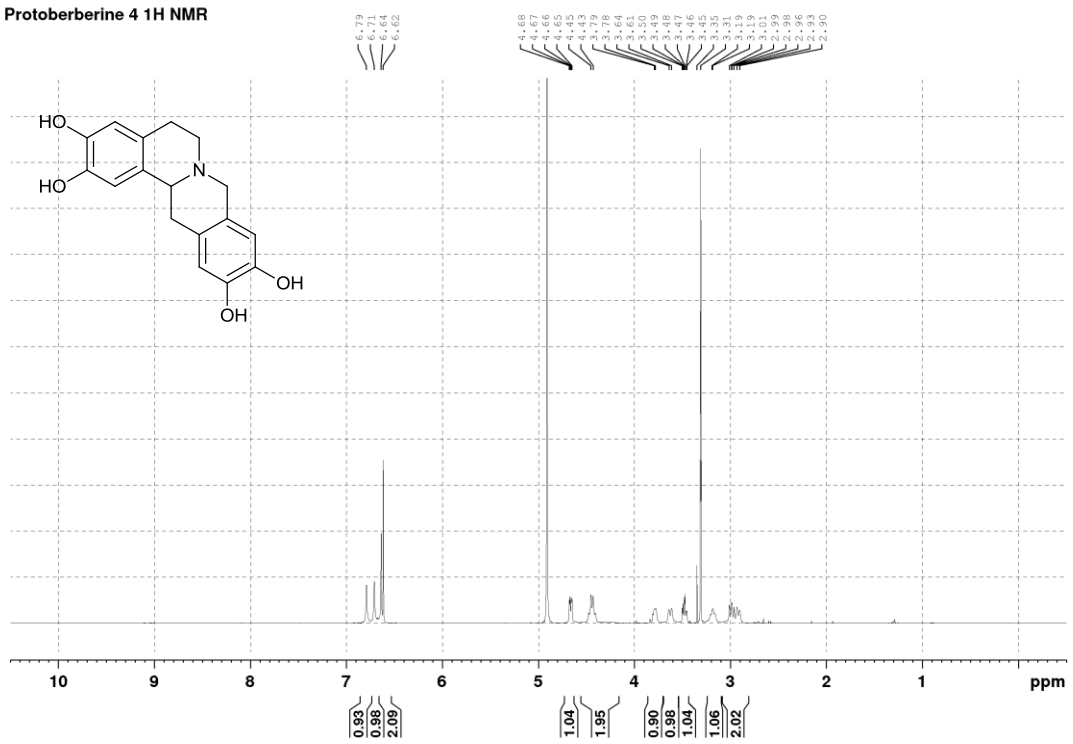
114.46
114.09
56.23
39.60
39.09
24.85
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

4


Protoberberine 4 1H NMR
HO
HO
N
OH
OH
6.79
6.71
6.64
6.62
4.68
4.67
4.66
4.65
4.45
4.43
3.79
3.78
3.64
3.61
3.50
3.49
3.48
3.47
3.46
3.45
3.35
3.31
3.19
3.01
2.99
2.98
2.96
2.93
2.90
10
9
8
7
6
5
4
3
2
1
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0.93
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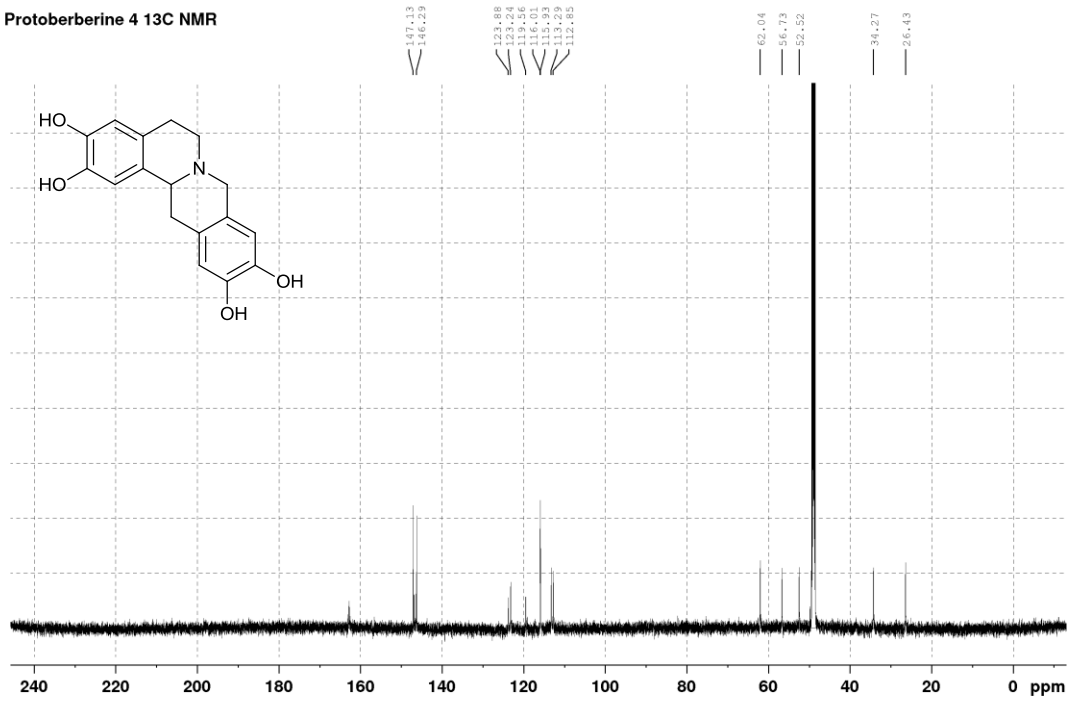
1.95
0.90
0.98
1.04
1.06
2.02


Protoberberine 4 13C NMR
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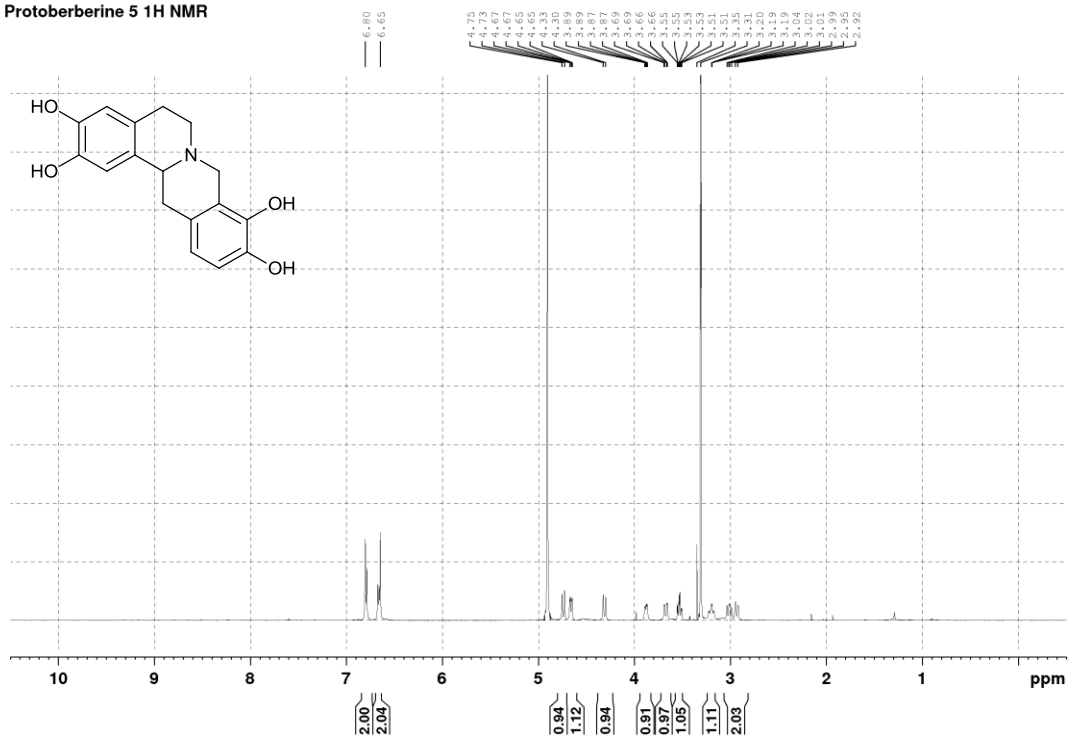
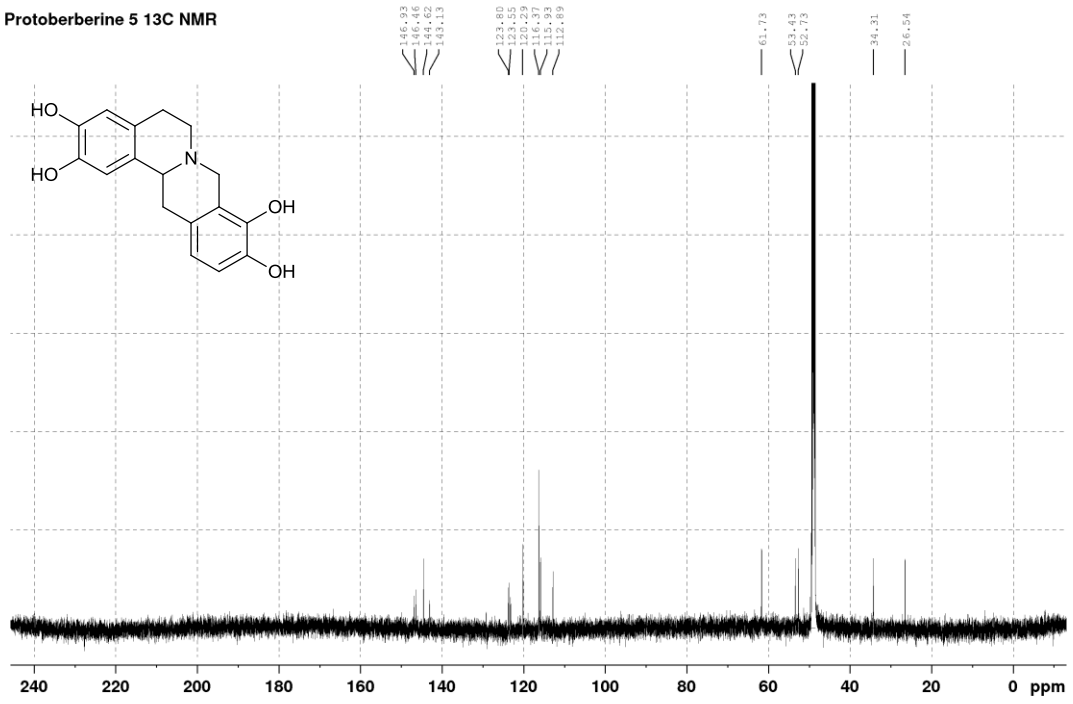
HO
N
OH
OH
147.13
146.29
123.88
123.24
119.56
116.01
115.93
113.29
112.85
62.04
56.73
52.52
34.27
26.43
240
220
200
180
160
140
120
100
80
60
40
20
0
ppm

5

Protoberberine 5 1H NMR

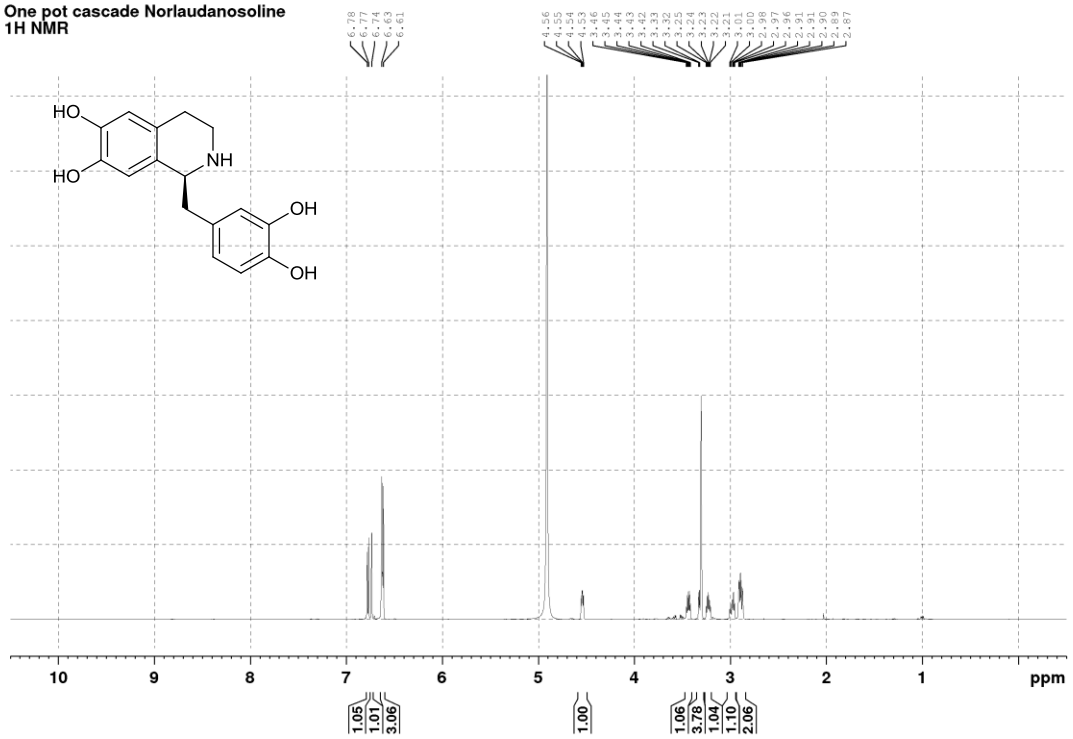


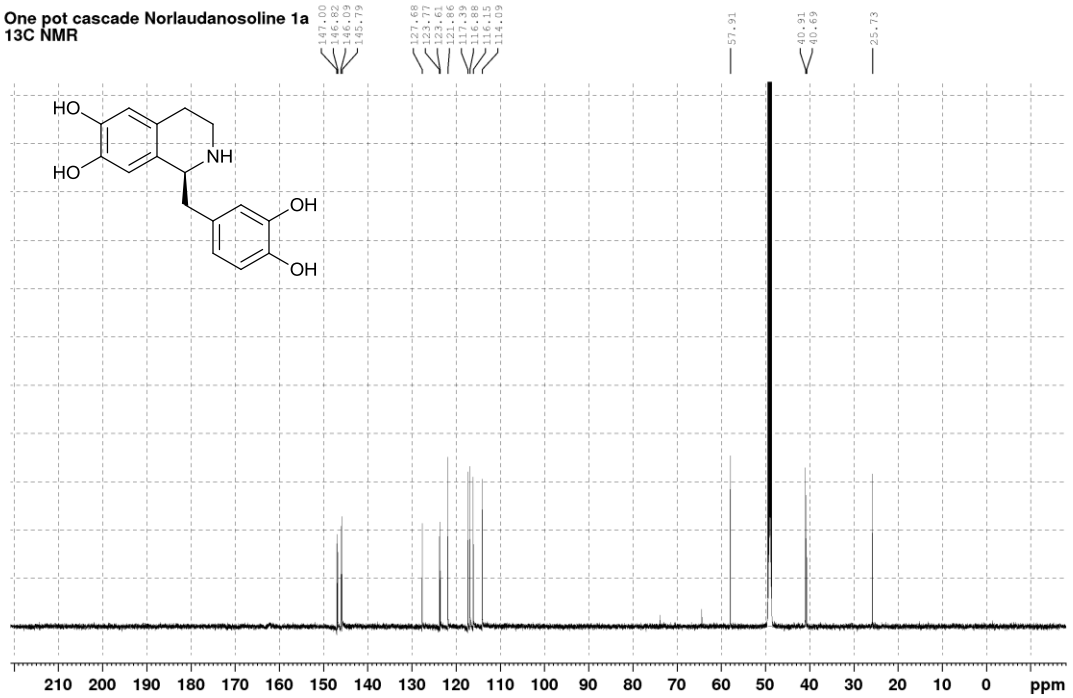
Protoberberine 5 13C NMR



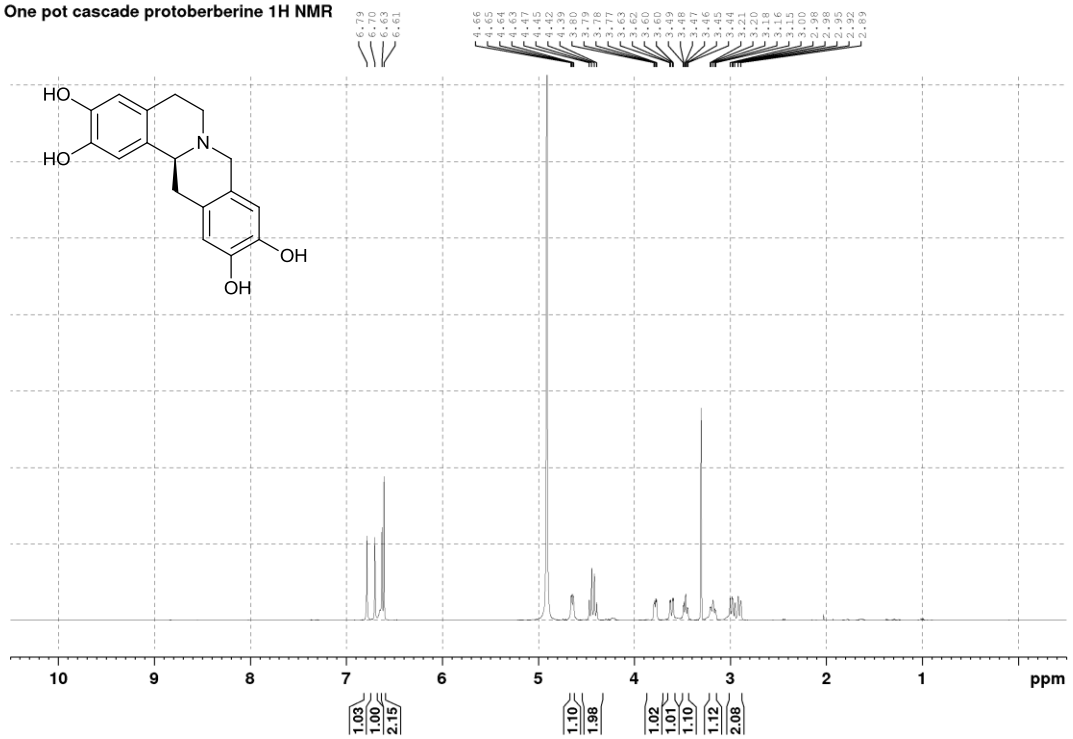
NMRs of enzymatic cascade products

1a





4


One pot cascade protoberberine 1H NMR
HO
HO
N
OH
OH
6.79
6.70
6.63
6.61
4.66
4.65
4.64
4.64
4.47
4.45
4.42
4.39
3.80
3.79
3.78
3.77
3.63
3.62
3.60
3.49
3.48
3.47
3.46
3.45
3.44
3.21
3.20
3.18
3.16
3.15
3.00
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2.89
10
9
8
7
6
5
4
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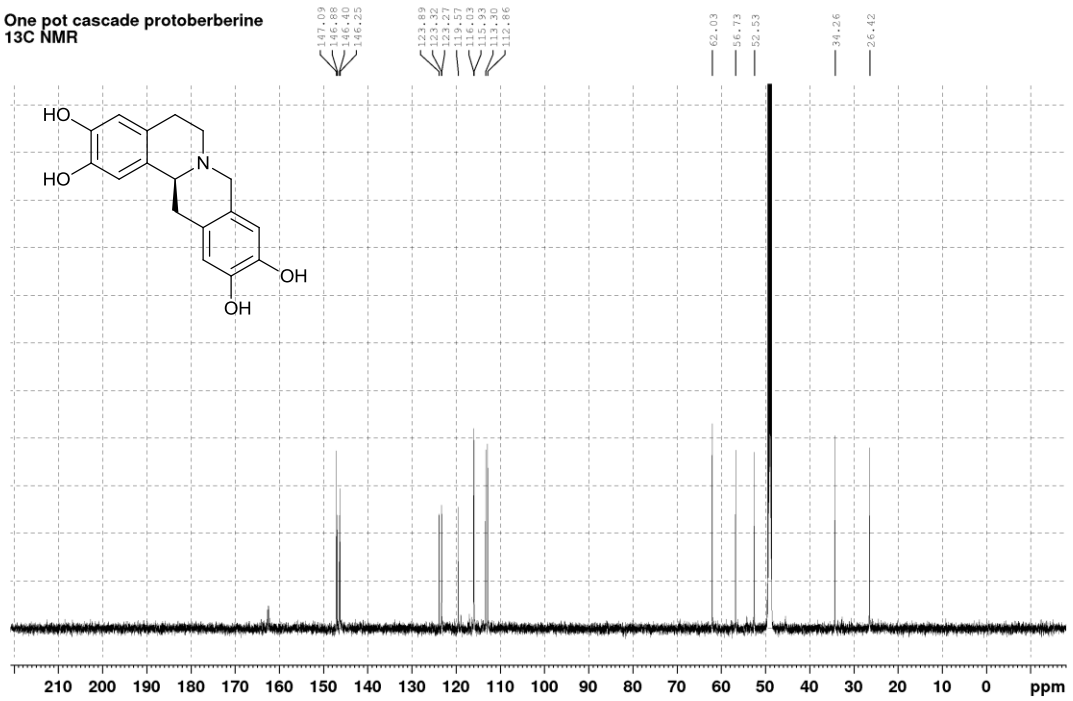
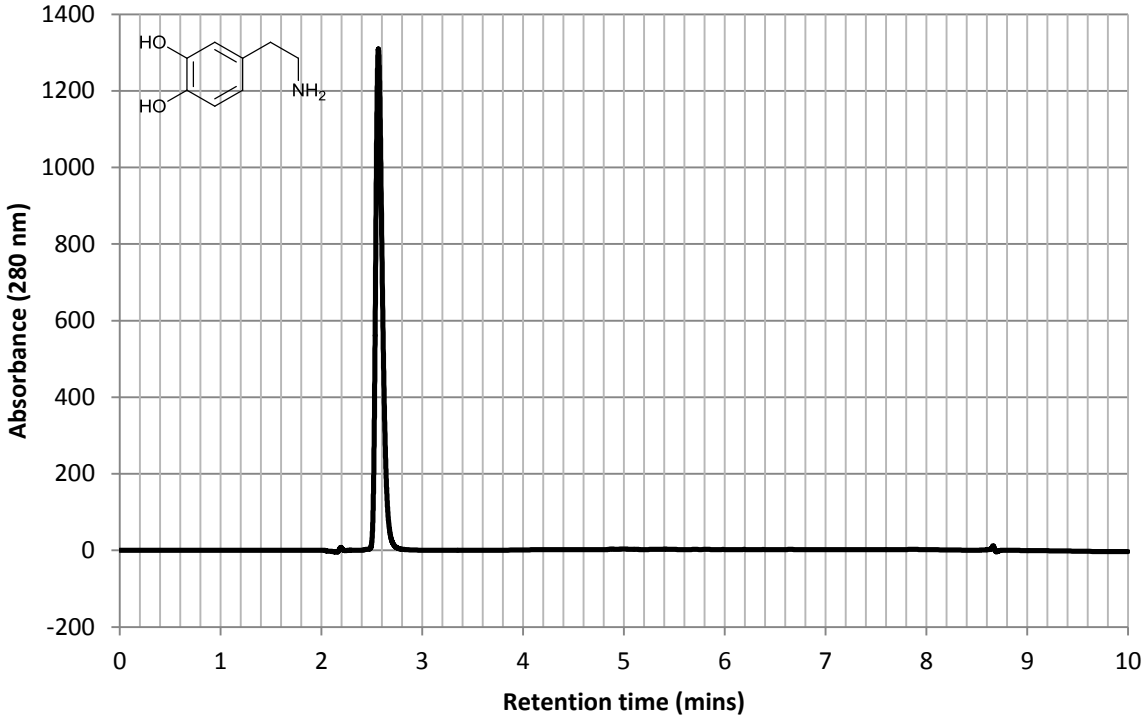
1.98
1.02
1.01
1.10
1.12
2.08


One pot cascade protoberberine
13C NMR
HO
HO
N
OH
OH
147.09
146.88
146.40
146.25
123.89
123.32
123.27
119.57
116.03
115.93
113.30
112.86
62.03
56.73
52.53
34.26
26.42
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

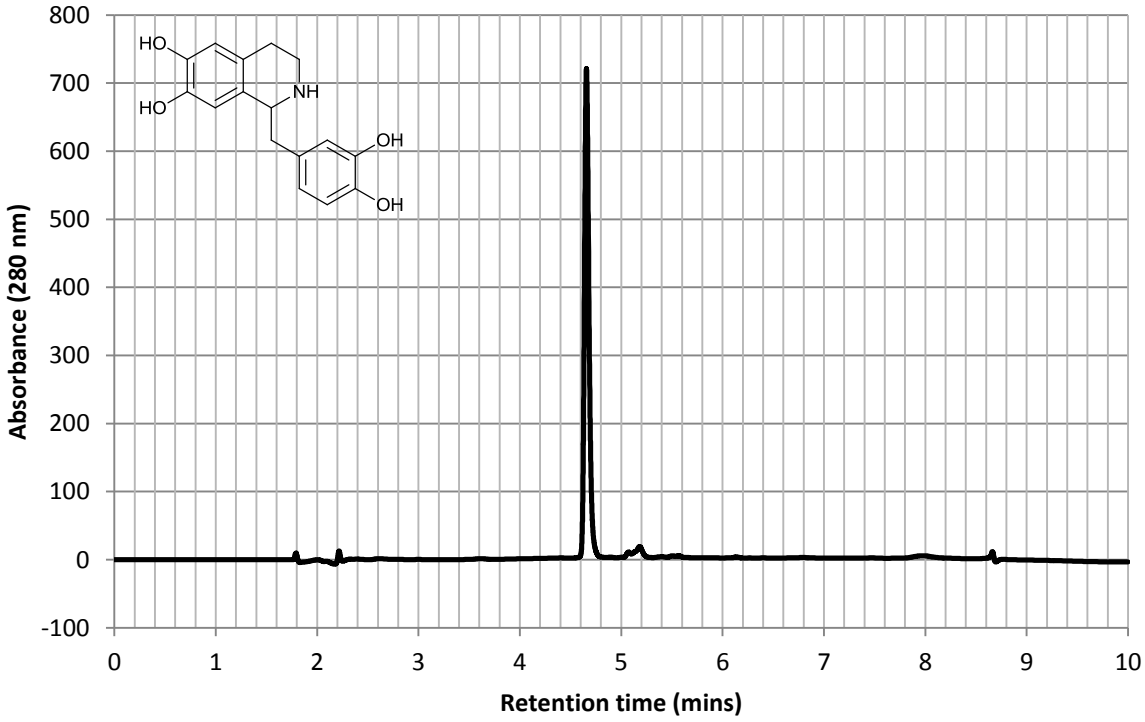
Analytical HPLC chromatograms

HPLC method 1

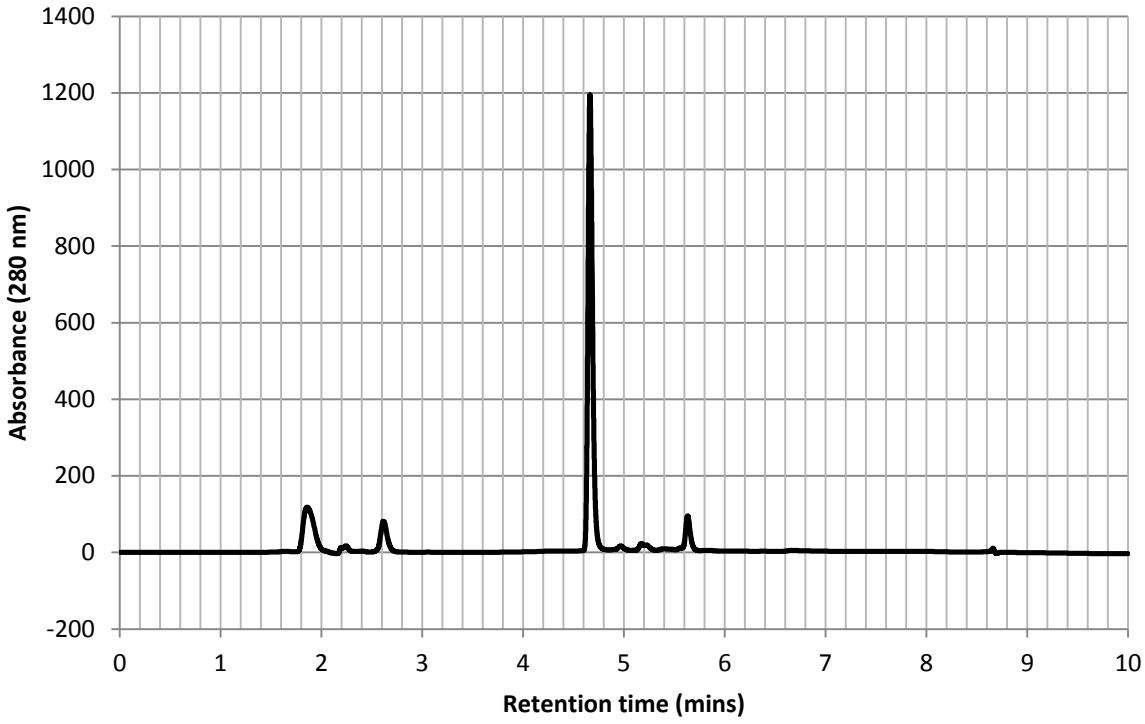
Dopamine 2a: chemical standard



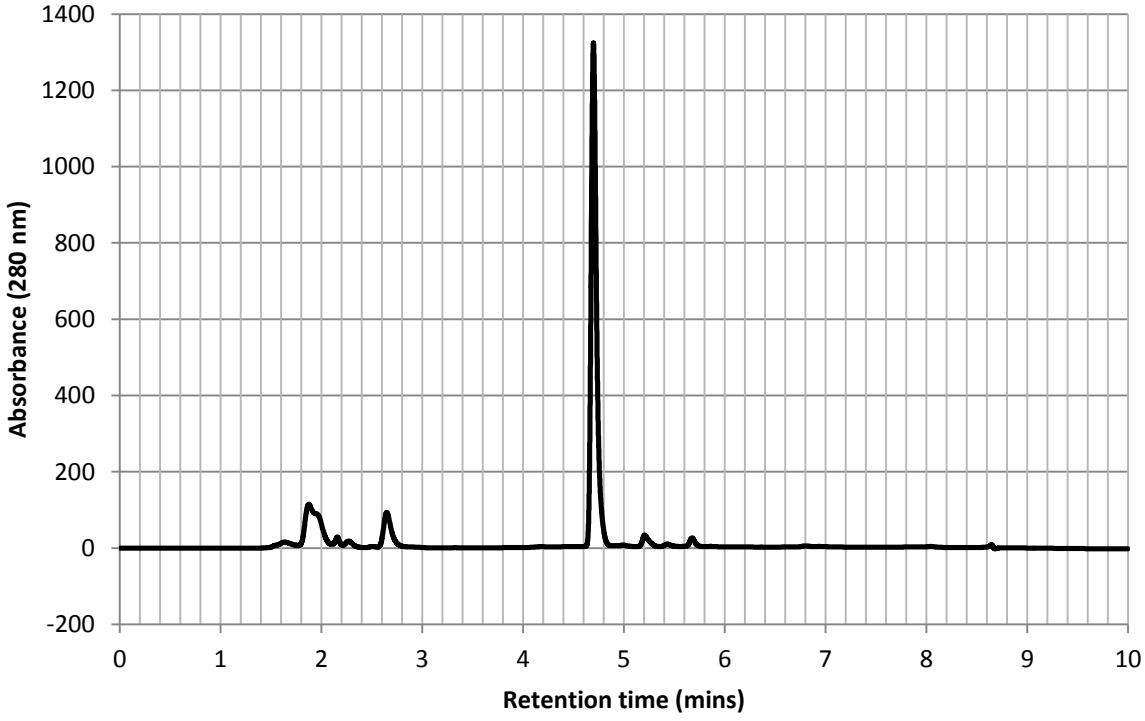
1a: chemical standard



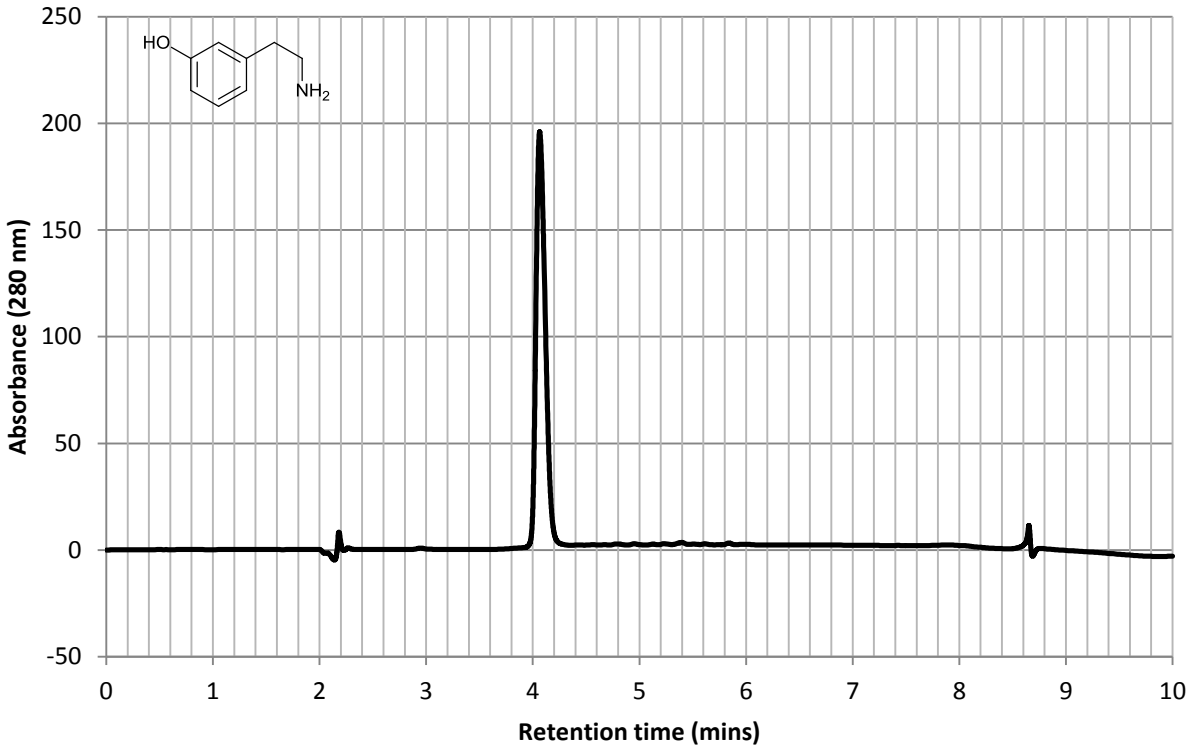
(*S*)-1a: small scale enzymatic synthesis



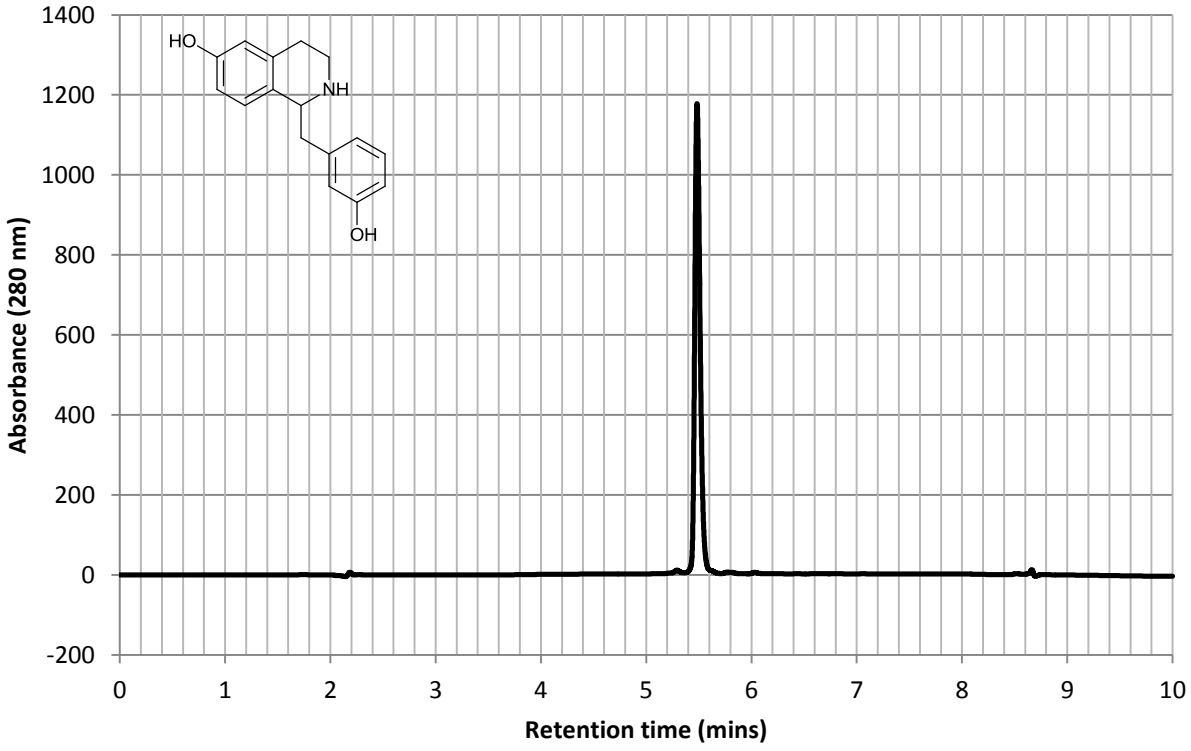
(*S*)-1a: preparatory scale enzymatic synthesis



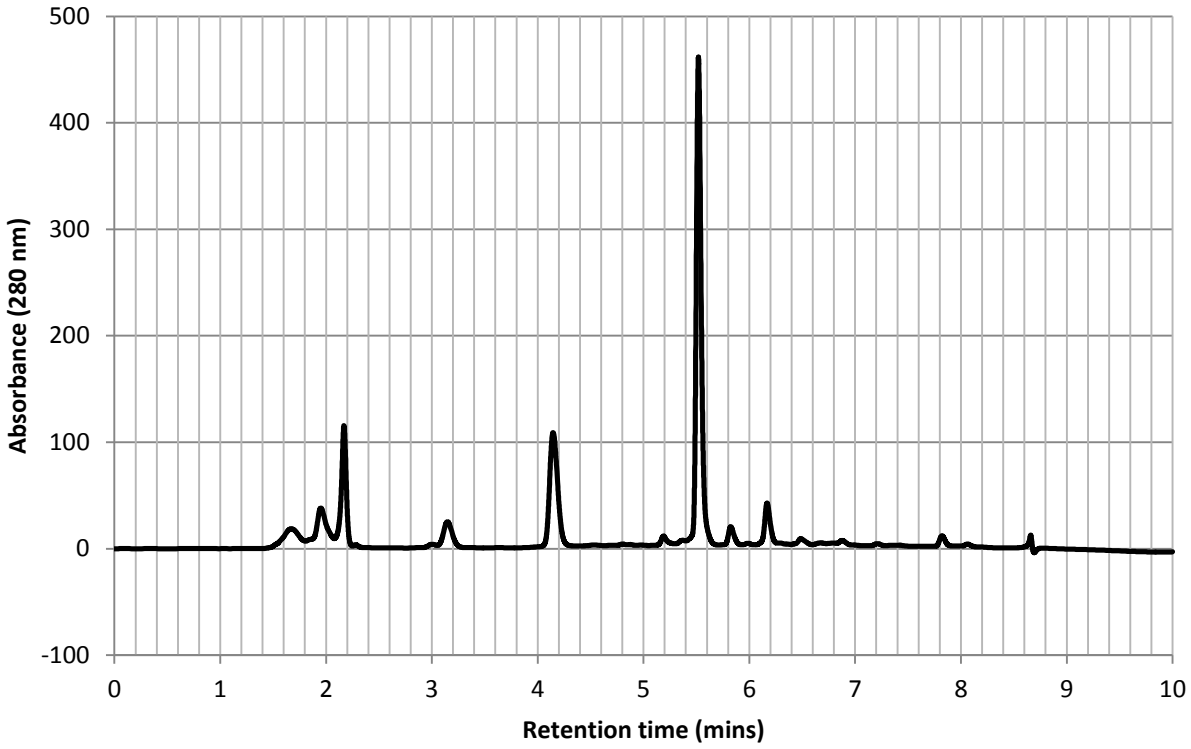
2b: chemical standard



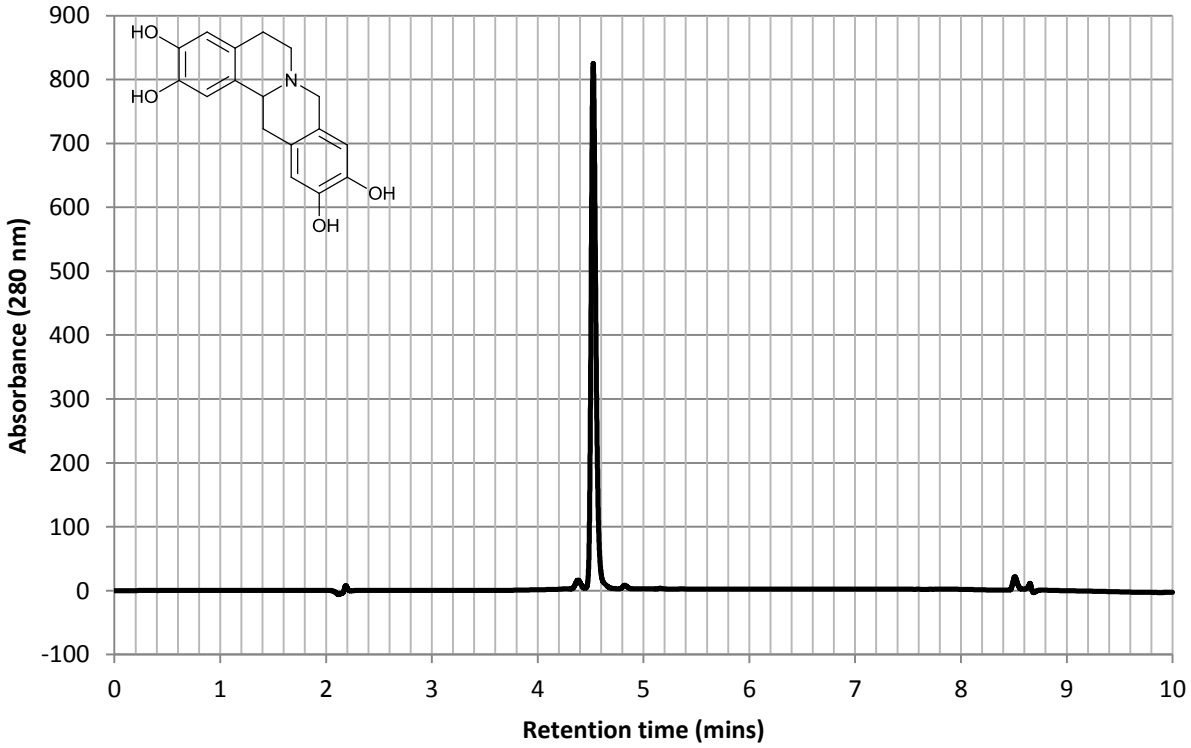
1b: chemical standard



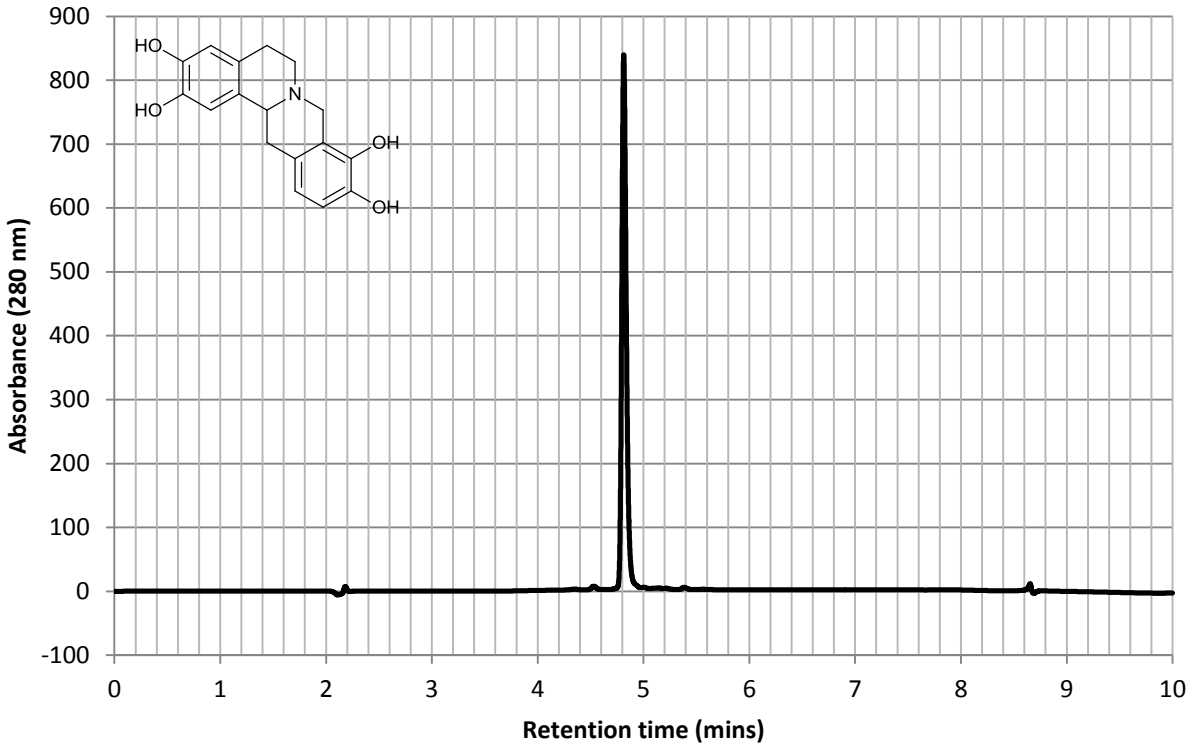
(*S*)-1b: enzymatic synthesis



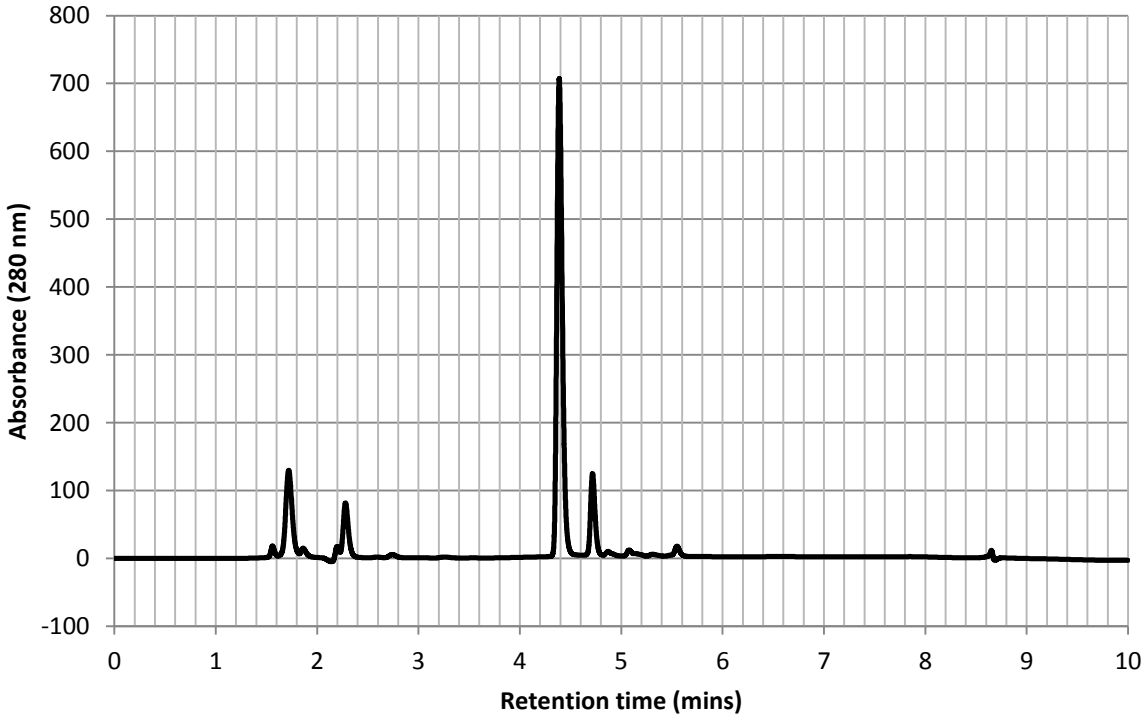
4: chemical standard



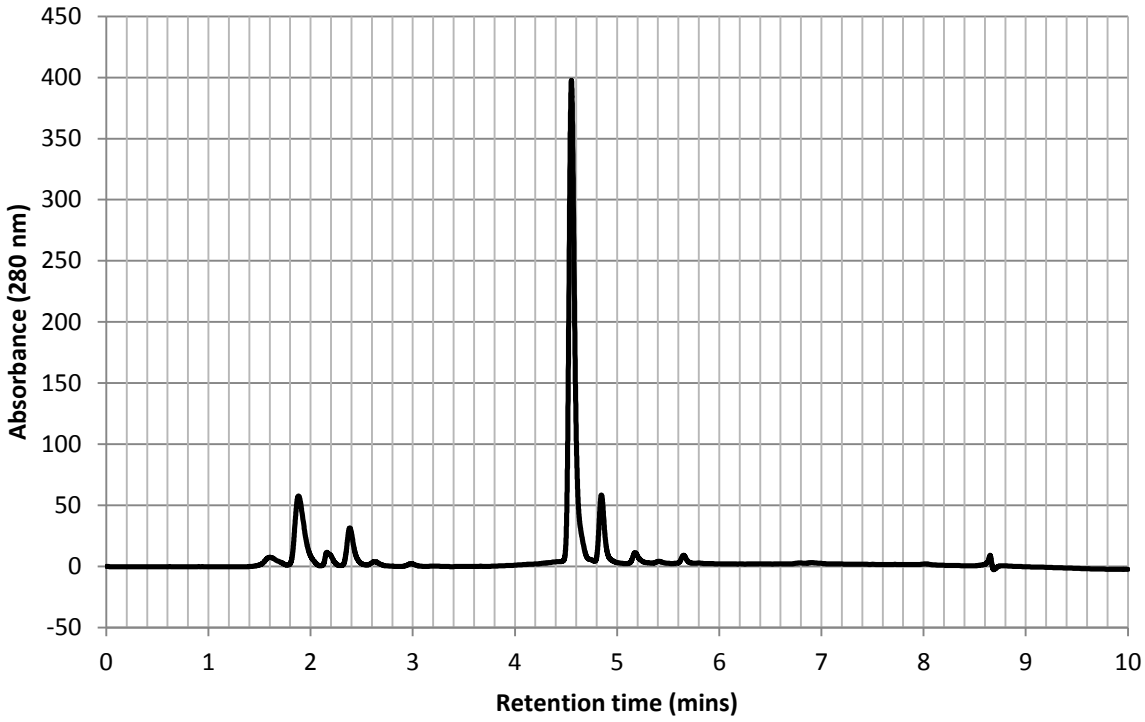
5: chemical standard



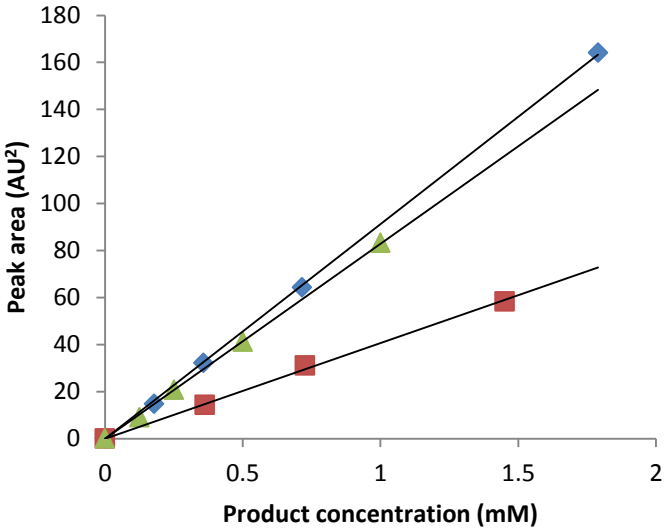
(*S*)- 4 and 5: small scale enzymatic cascade



(*S*)- 4 and 5: prep scale enzymatic cascade



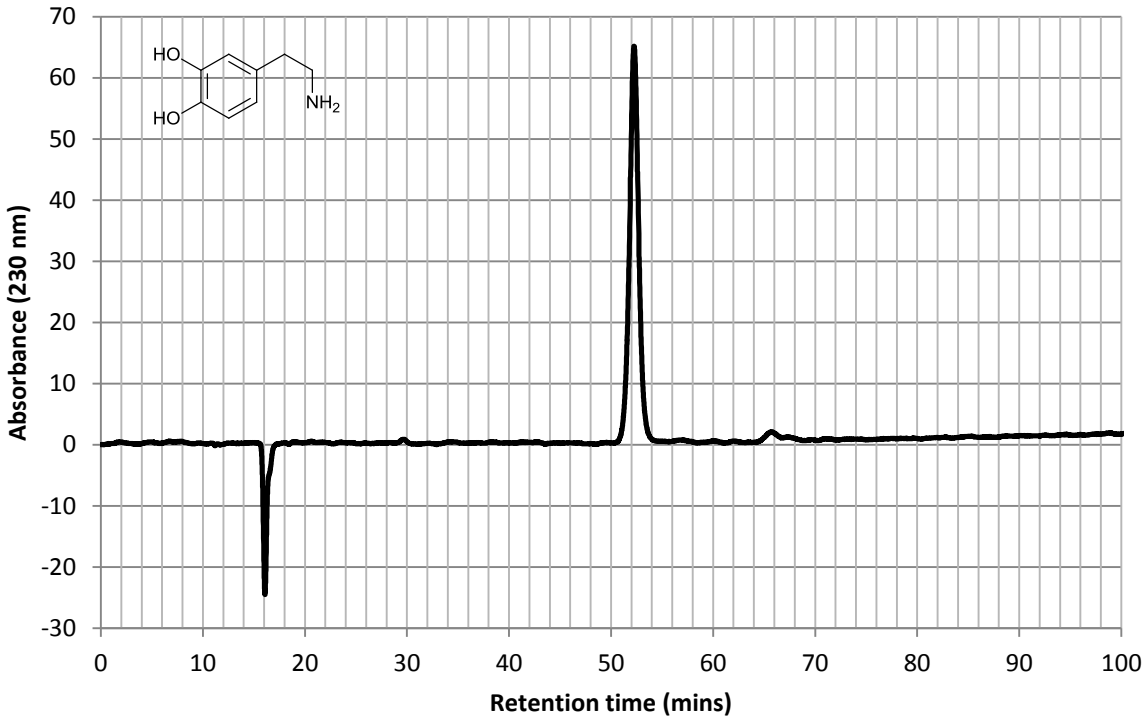
Standard curves



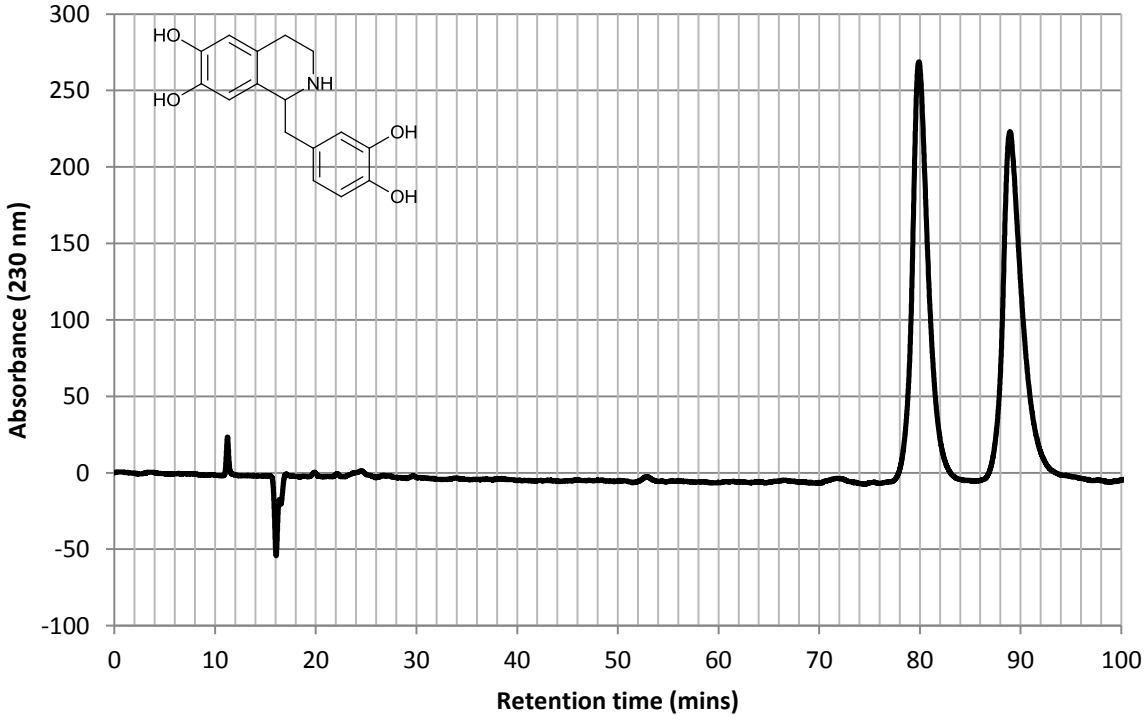
Peak area of chemical standards at 280 nm. **1a** is blue diamonds (gradient = 95.5), **2a** is yellow triangles (gradient = 40.6) and **4** is red squares (gradient = 111)

Chiral HPLC chromatograms

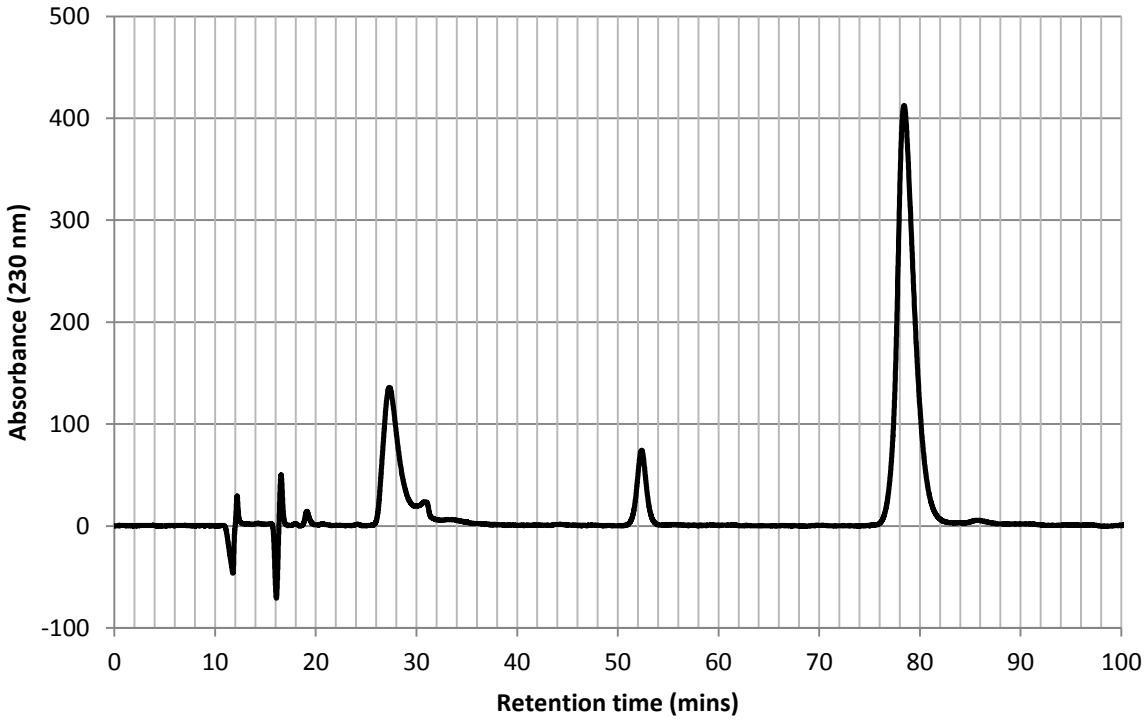
Dopamine 2a: chemical standard, HPLC method 2.



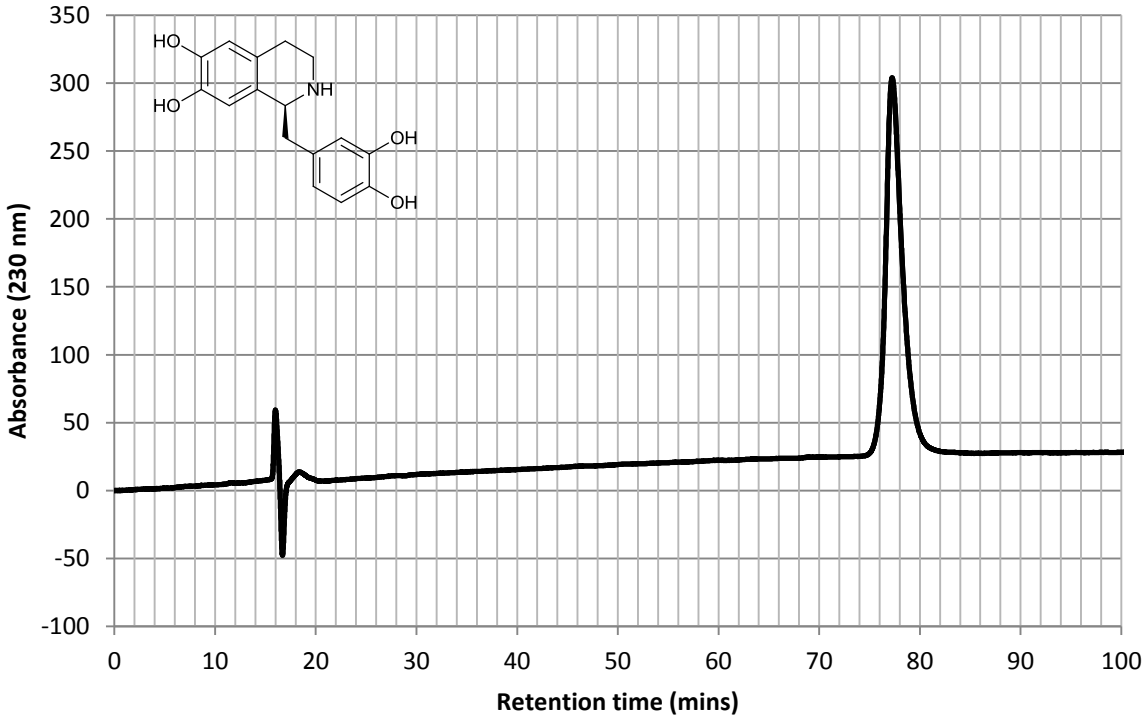
***rac*-1a**: chemical standard, HPLC method 2.



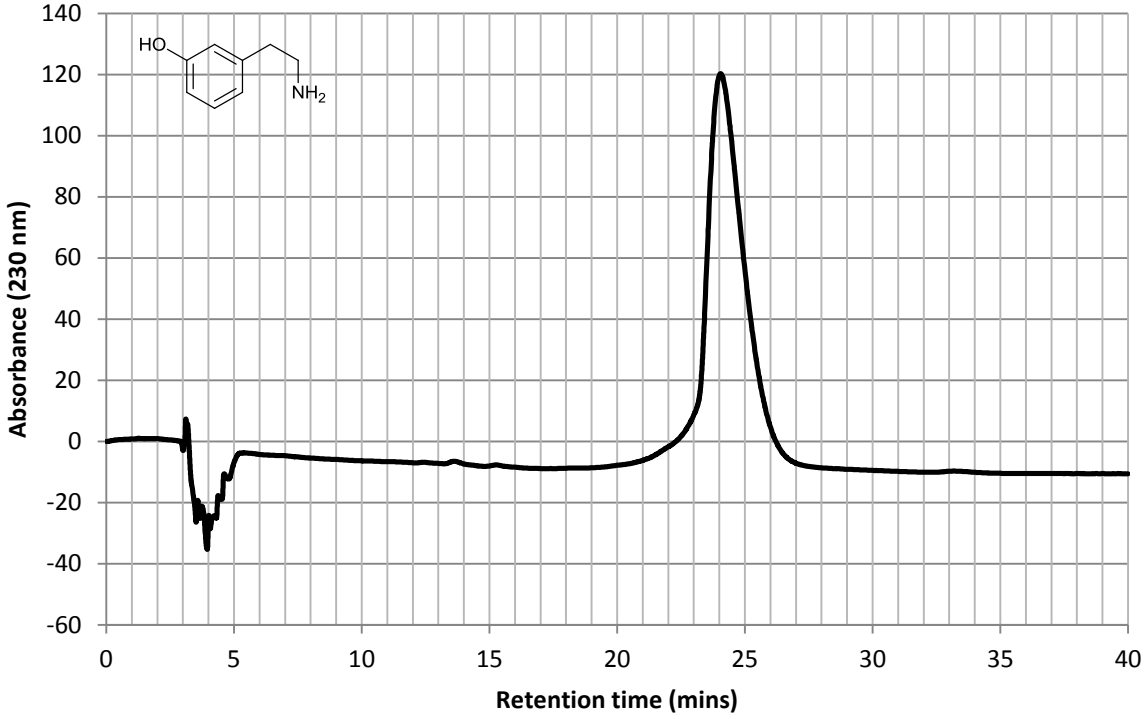
(*S*)-1a: crude, small scale enzymatic cascade, HPLC method 2.



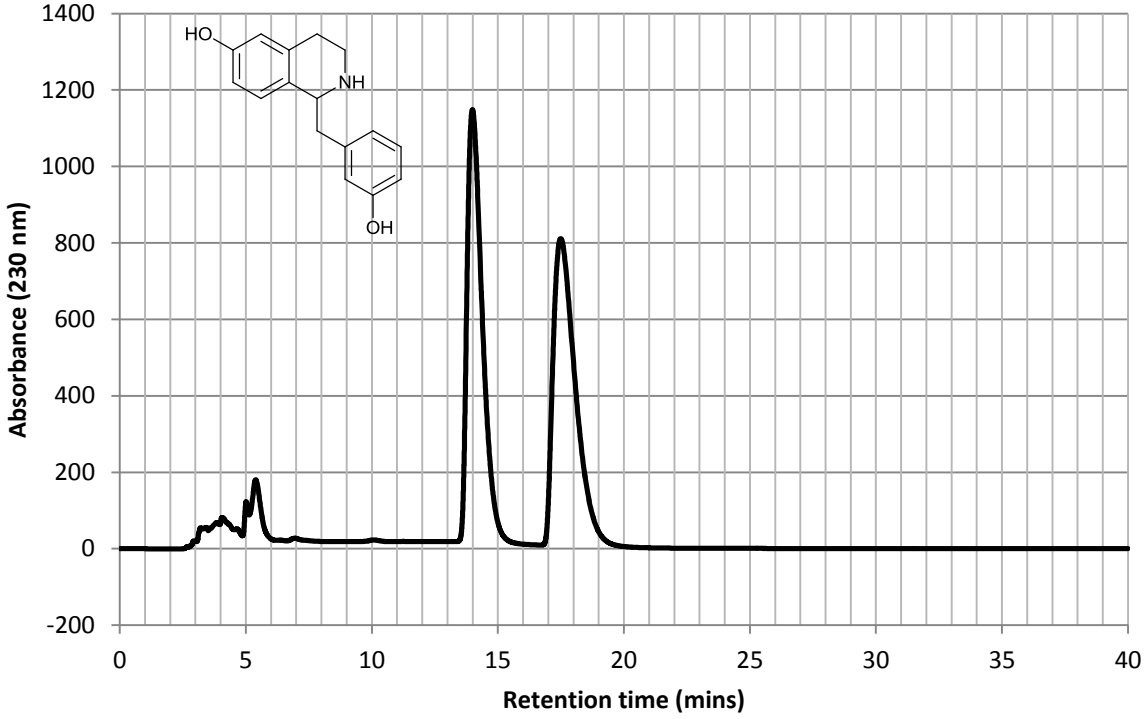
(*S*)-1a: purified, prep scale enzymatic synthesis, HPLC method 2.



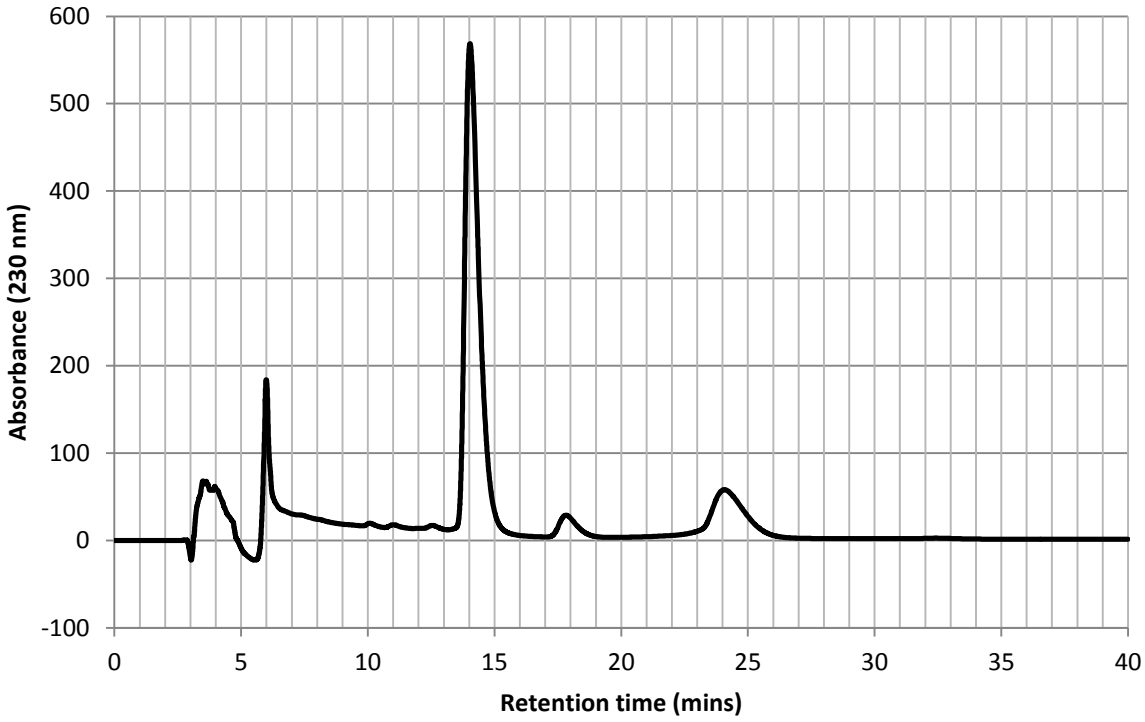
2b: chemical standard, method 3.



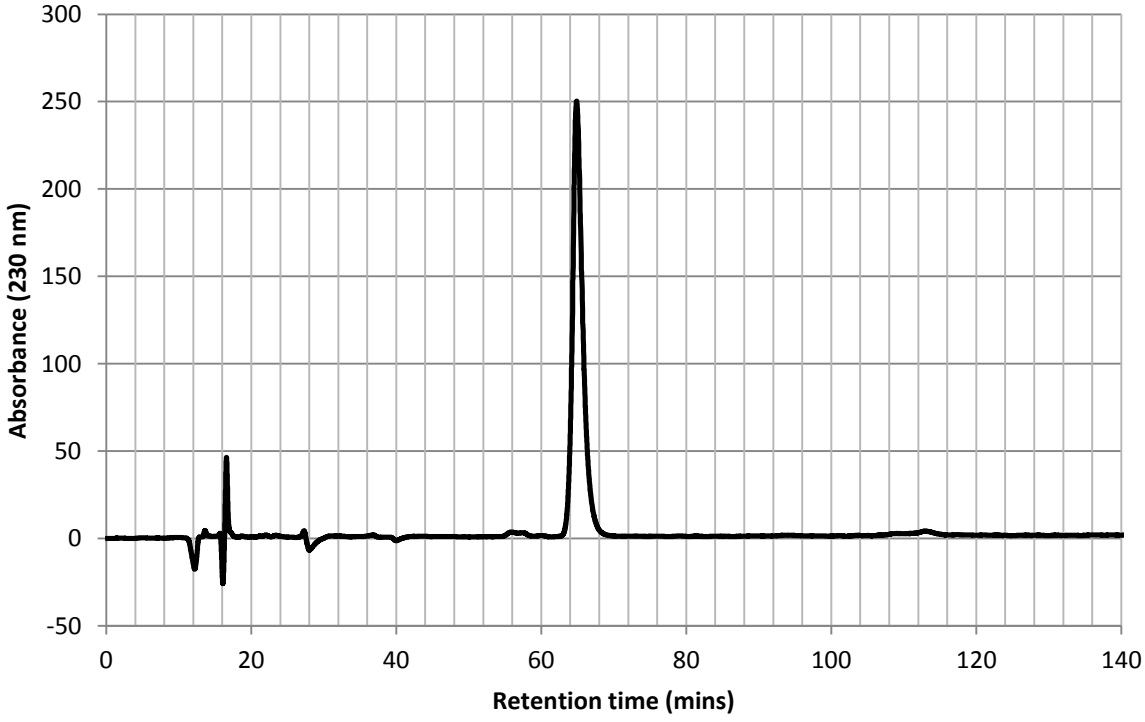
***rac*-1b**: chemical standard, HPLC method 3.



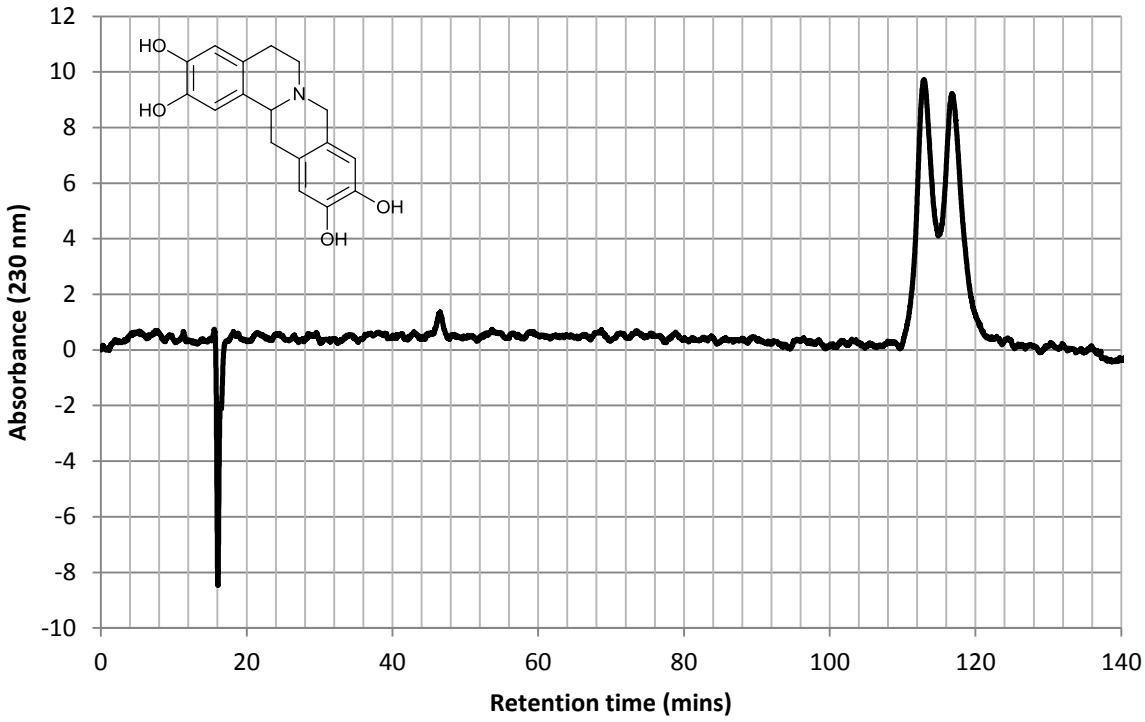
(*S*)-1b: crude, enzymatic synthesis, HPLC method 3.



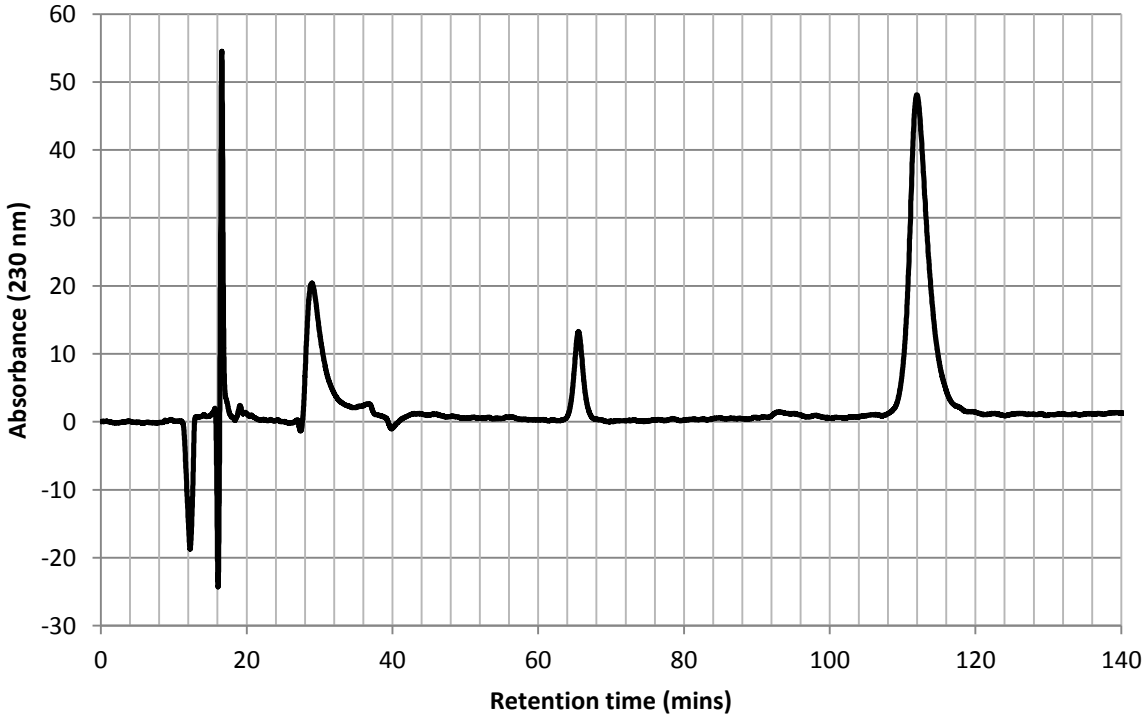
6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline: crude, unverified chemical standard (10 mM **2a,** 20 mM formaldehyde, 0.5 M sodium phosphate pH 6, 37 °C, 30 min), HPLC method 2.



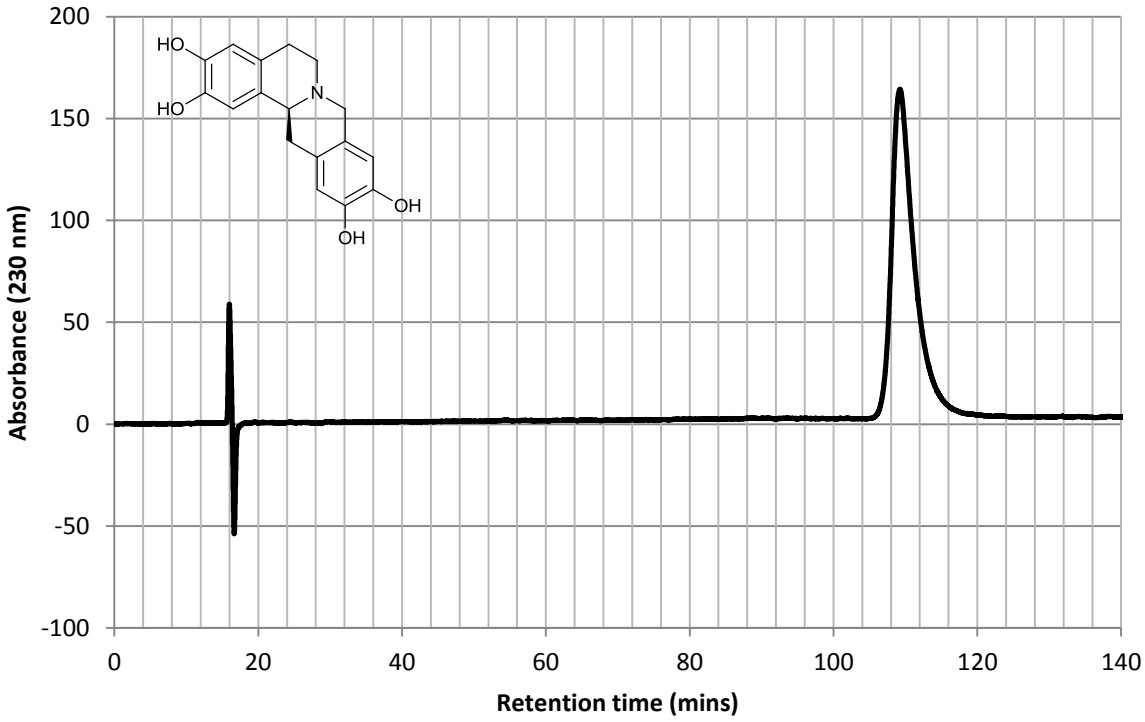
***rac*-4:** chemical standard, HPLC method 2.



(*S*)-4: crude, small scale enzymatic cascade, HPLC method 2.



(*S*)-4: purified, large scale enzymatic cascade, HPLC method 2.



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