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1 Chemical Biology

1.1 General methods

Thermomixers used were a BIOER Mixing Block MB-102 and a Stuart Orbital Incubator SI600.

Centrifuges used were an Eppendorf Centrifuge 5415R, Eppendorf Centrifuge 5810R and Beckman Coulter Avanti JXN-26.

Autoclaves used were a Priorclave TACTROL 2 and a Priorclave TACTROL 3. Where sterilisation of waste and media was performed, the temperature was held at 121 °C for 30 min.

The **Sonicator** used was a Branson Sonifier 150 with microprobe tip at a power of 13 W.

Potassium phosphate buffer (100 mM, pH 8.0): 820 mg K₂HPO₄ and 41 mg KH₂PO₄ made up to 50 mL in distilled water.

Terrific broth (TB) 47.6 g of TB and 4 mL glycerol in 1 L distilled water was then autoclaved.

1.2 Protein expression

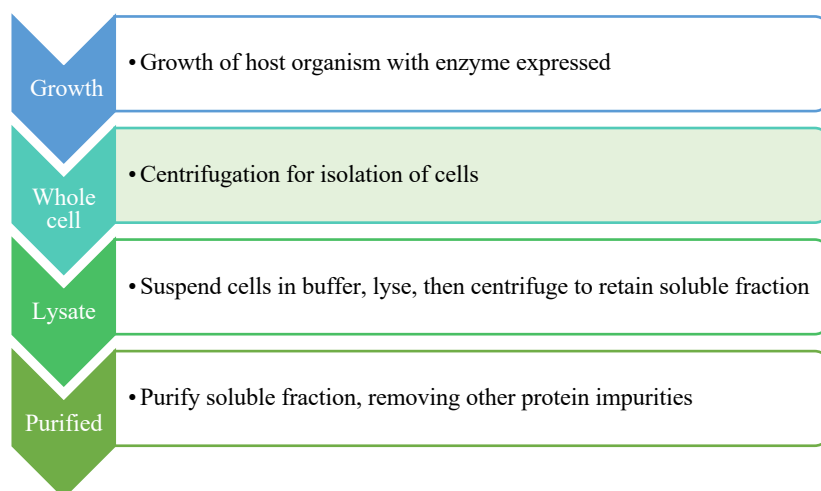


Figure 1: Typical steps for producing biocatalysts, which can be used in whole cell, lysate or purified form. Here, whole cells were reacted in a mixer mill which can lyse cells in situ.

Overnight cultures (10 mL) of *Cv*-TAm¹ in *E. coli* from the UCL TAM library were prepared in TB, supplemented with kanamycin (50 µg/mL), and incubated overnight at 37 °C. Cells were subcultured using 1% v/v inoculum in 2 L shake flasks containing 500 mL of the same supplemented broth at 37 °C and 250 rpm. Transaminases were induced with 1 mM of IPTG when growing in the early exponential phase (OD₆₀₀ = 0.6 to 0.8), and the temperature was dropped to 25 °C overnight until harvesting. Cells were harvested by centrifugation and stored at -80 °C. When needed, the cell pellet was resuspended in potassium phosphate (KPi) buffer (100 mM, pH 8.0) containing 2 mM PLP at a 1:25 volume ratio

(1 mL of the resuspension buffer per 25 mL of original cell suspension before harvesting). For whole cell reactions, these were lyophilised until dry and stored at -20 °C before being used in reactions.

For clarified lysates, they were sonicated on ice using 10 cycles of 10 s on, 10 s off at 13 W. The sonicated suspension was centrifuged at 12,000 rpm at 4 °C for 30 min to obtain the clarified lysate.

To calculate the total protein concentration, *Cv*-TAm whole cells were lysed and the Bradford method² was used. This was found to be 0.15 mg protein per mg of (lyophilised) whole cell. Analysis of the SDS page gel using ImageJ showed that the proportion of *Cv*-TAm was 31% (0.05 mg per mg of whole cell).

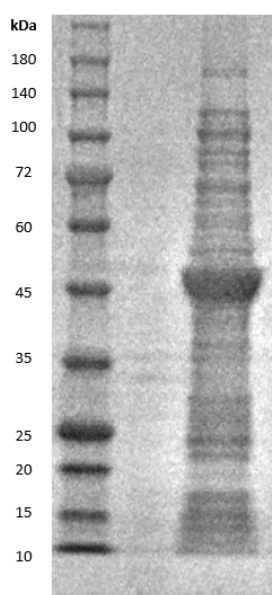


Figure 2: SDS-page gel of *Chromobacterium violaceum* (*Cv*-TAm).¹ Protein ladder: Trident Blue Prestained Protein Ladder¹ from GeneTex (MW 10 to 180 kDa).

1.3 Assays

The assay was performed with 2 × 5 mm stainless steel balls in 5 mL stainless steel jars (all from Retsch) containing the substrate (50 mM or 100 mM), isopropylamine hydrochloride (IPA.HCl) (3.75 M), PLP (0.5 mM), KPi buffer (100 mM, pH 8.0, 2 mL total volume) and lyophilised whole cell *Cv*-TAm. The reaction was placed in a Retsch MM500 vario mixer mill (Verder Scientific UK Ltd) and shaken at 25 Hz for 30 min then aged for 30 min (i.e. 0 Hz at rt) and this was repeated twice (total reaction time 2 h). The jars were rinsed with water and acetonitrile (containing 0.1% TFA). Insoluble material was removed by centrifugation (4 °C, 12000 rpm, 30 min) and the supernatant diluted with water and analysed by analytical HPLC. Yields were determined by HPLC against product standards (Table 1).

For reactions that were not milled, the reaction was carried out in Eppendorf tubes (2 mL) on a mixing block at room temperature for 30 min (300 rpm) then for 30 min at 0 rpm, repeated twice (total reaction time 2 h).

For no enzyme controls, the same mass of empty vector lyophilised BL21 whole cell was used instead of lyophilised *Cv*-TAm whole cell.

Table 1. The use of whole cell *Cv*-TAm under mechanochemical reaction conditions and using a thermomixer (no milling).

| aldehyde/ketone | Amine 1b-12b% yield (mg of lyophilised whole cell <i>Cv</i> -TAm used) | | |
|-----------------|--|--|---------------------------------|
| | Mechanoenzymatic (50 mM substrate) | Mechanoenzymatic (100 mM substrate) | No milling (50 mM substrate) |
| 1a | 96 (10) | 93 (20) | 20 (10) |
| 2a | 84 (10) | 88 (20) | 17 (10) |
| 3a | 77 (10) | 89 (20) | 13 (10) |
| 4a | 98 (10) | 92 (20) | 30 (10) |
| 5a | 89 (10) | 88 (20) | 20 (10) |
| 6a | 96 (10) | 94 (20) | 71 (10) |
| 7a | 63 (10) | 43 (20) | 7 (10) |
| 8a | 79 (40) | 54 (60) | 14 (40) |
| 9a | 90 (20) | 97 (20) | 27 (20) |
| 10a | 78 (20) | 75 (20) | 22 (20) |
| 11a | 58 (20) | 58 (20) | 25 (20) |
| 12a | 74 (30) | 33 (40) | 3 (30) |

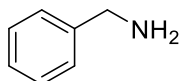
Yields of amines **1b-12b** when reacting aldehydes and ketones **1a-12a** (50 or 100 mM), PLP (0.5 mM), lyophilised whole cell *Cv*-TAm (0.05 mg TAm per mg whole cell enzyme) and IPA.HCl (3.75 M) in KPi buffer (pH 8.0, 100 mM, 2 mL total volume). **Ball mill:** This was performed in 5 mL stainless steel jars with 2 × 5 mm stainless steel balls, with shaking at 25 Hz for 2 cycles of 30 min shaking, then 30 min aging at rt. Control reactions were carried out using whole cell empty vector BL21 which gave no amine products in all cases. **No milling:** This was performed in Eppendorf tubes in a thermomixer at rt for 2 h (2 cycles, 30 min mixing, 30 min standing). Yields were determined by HPLC against product standards.

2 Analytical HPLC and GC

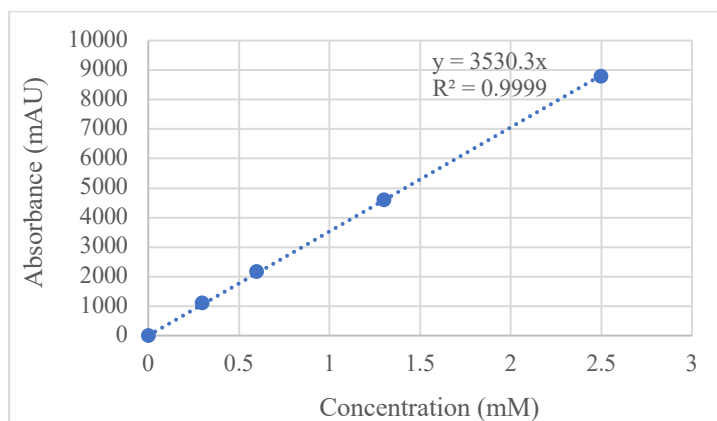
2.1 Achiral HPLC

Achiral HPLC was performed on an Agilent 1260 Infinity system using an ACE 5-C18-AR column (150 × 4.6 mm).

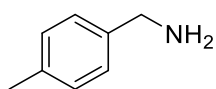
Benzylamine 1b



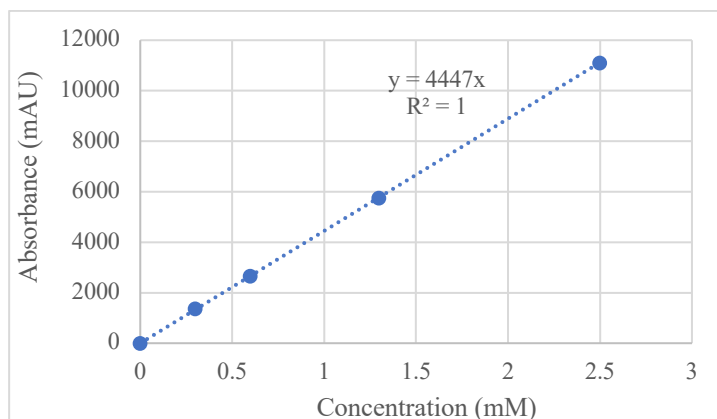
The concentration of benzylamine was determined with UV detection at 210 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The benzylamine eluted at a retention time of 5.3 min. Results were verified in duplicate.



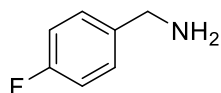
4-Methylbenzylamine 2b



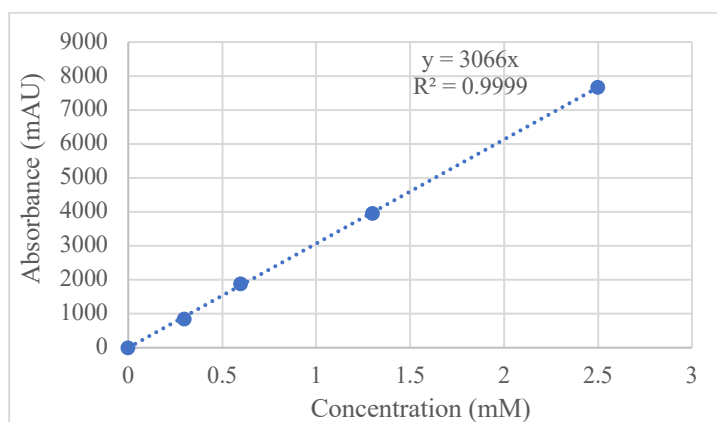
The concentration of 4-methylbenzylamine was determined with UV detection at 210 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 4-methylbenzylamine eluted at a retention time of 6.6 min. Results were verified in duplicate.



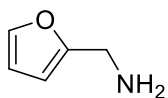
4-Fluorobenzylamine 3b



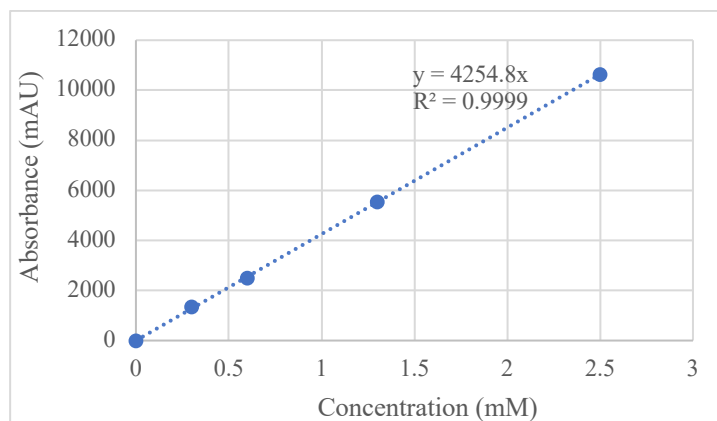
The concentration of 4-fluorobenzylamine was determined with UV detection at 210 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 4-fluorobenzylamine eluted at a retention time of 5.9 min. Results were verified in duplicate.



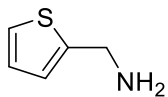
Furfurylamine 4b



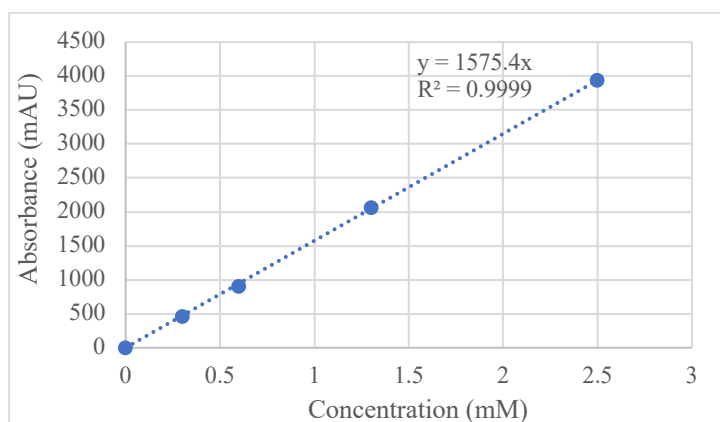
The concentration of furfurylamine was determined with UV detection at 210 nm using a linear gradient 3-30% B over 10 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The furfurylamine eluted at a retention time of 3.2 min. Results were verified in duplicate.



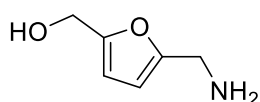
2-Thiophenemethylamine 5b



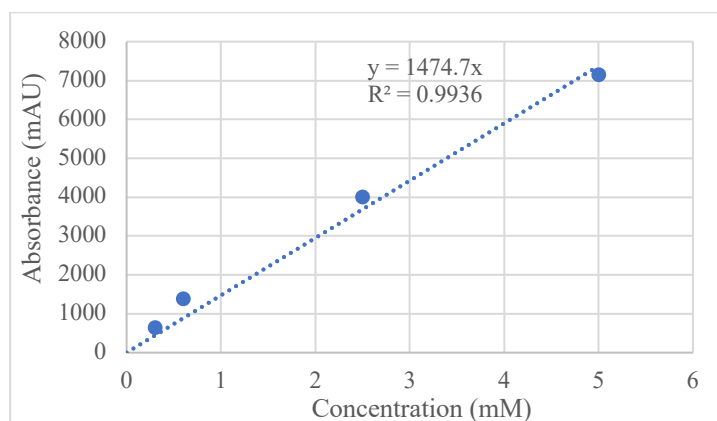
The concentration of 2-thiophenemethylamine was determined with UV detection at 210 nm using a linear gradient 3-30% B over 10 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 2-thiophenemethylamine eluted at a retention time of 5.1 min. Results were verified in duplicate.



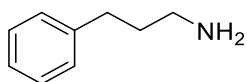
(5-Aminomethyl-furan-2-yl)-methanol 6b



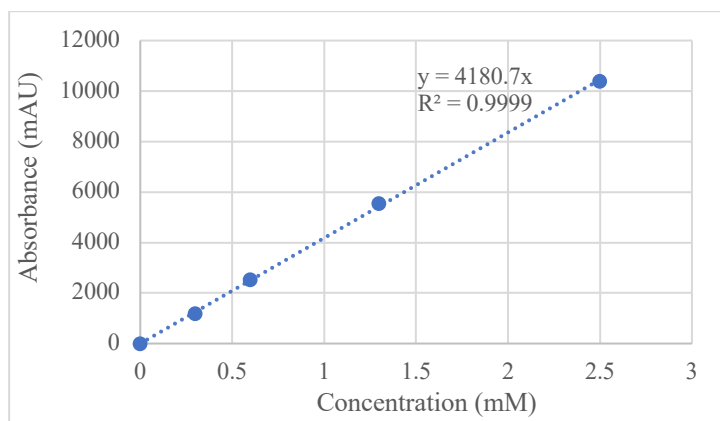
The concentration of (5-aminomethyl-furan-2-yl)-methanol was determined with UV detection at 210 nm using a linear gradient 3-30% B over 10 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The (5-aminomethyl-furan-2-yl)-methanol eluted at a retention time of 2.9 min. A quantitative NMR study calculated the amine purity as 37.5% therefore yields were adjusted accordingly. Results were verified in duplicate.



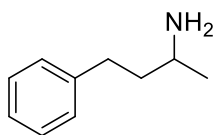
3-Phenylpropylamine 7b



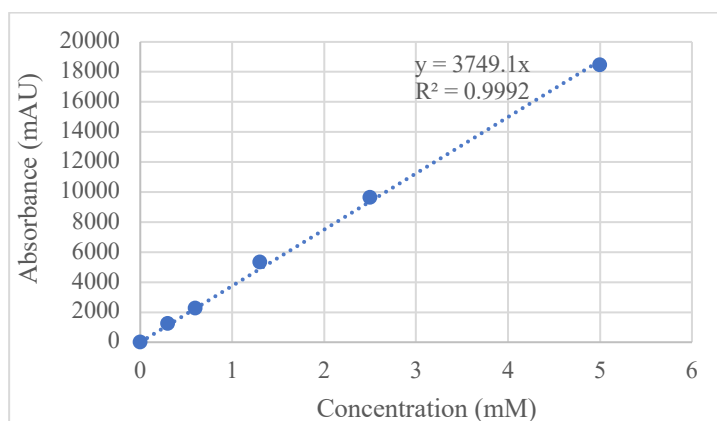
The concentration of 3-phenylpropylamine was determined with UV detection at 210 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 3-phenylpropylamine eluted at a retention time of 7.4 min. Results were verified in duplicate.



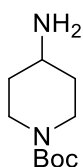
4-Phenylbutan-2-amine 8b



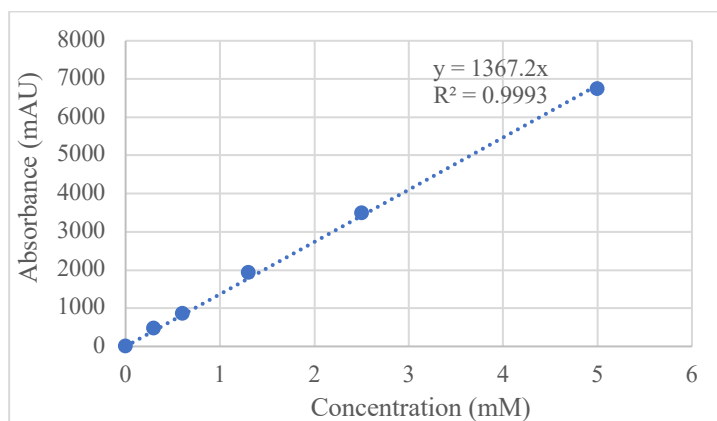
The concentration of 4-phenylbutan-2-amine was determined with UV detection at 210 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 4-phenylbutan-2-amine eluted at a retention time of 7.9 min. Results were verified in duplicate.



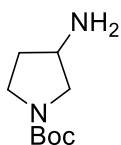
4-Amino-1-Boc-piperidine 9b



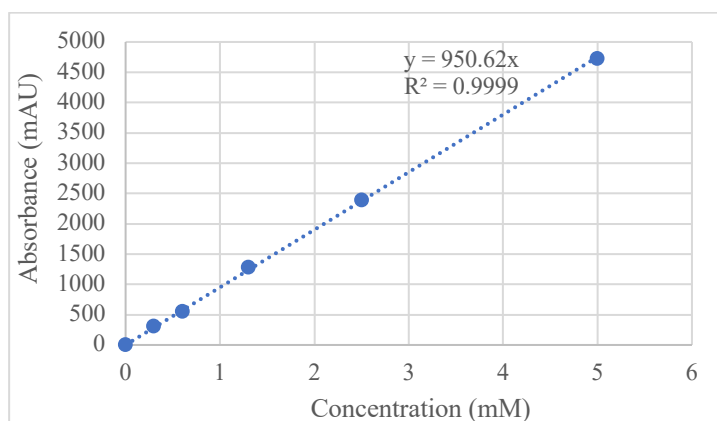
The concentration of 4-amino-1-Boc-piperidine was determined with UV detection at 204 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 4-amino-1-Boc-piperidine eluted at a retention time of 7.2 min. Results were verified in duplicate.



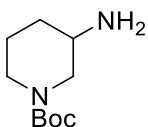
3-Amino-1-Boc-pyrrolidine 10b



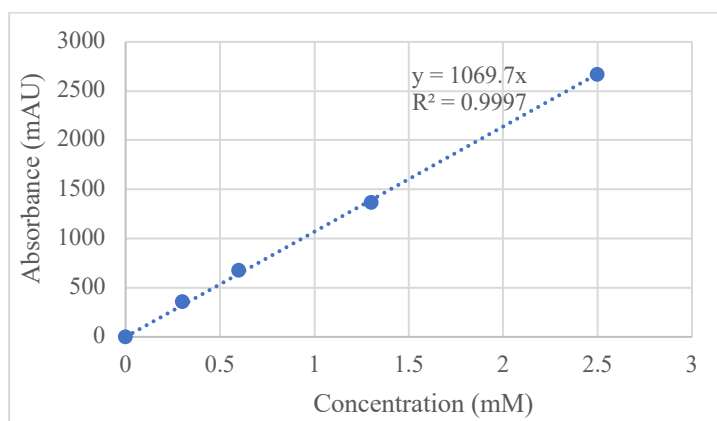
The concentration of 3-amino-1-Boc-pyrrolidine was determined with UV detection at 204 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 3-amino-1-Boc-pyrrolidine eluted at a retention time of 6.8 min. Results were verified in duplicate.



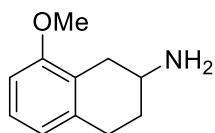
3-Amino-1-Boc-piperidine 11b



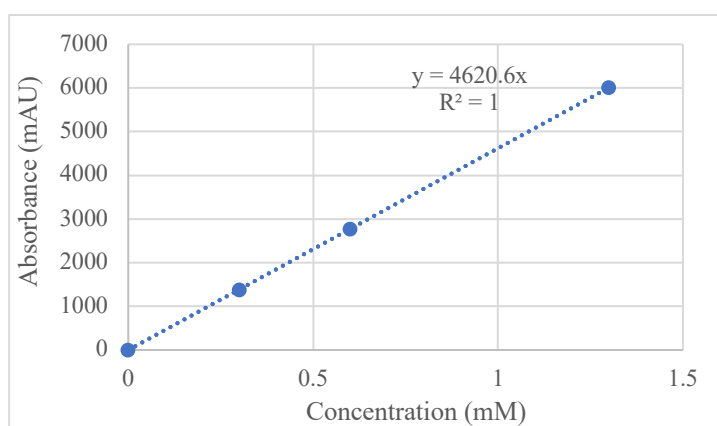
The concentration of 3-amino-1-Boc-piperidine was determined with UV detection at 204 nm using a linear gradient 5-95% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 3-amino-1-Boc-piperidine eluted at a retention time of 7.3 min. Results were verified in duplicate.



8-Methoxy-1,2,3,4-tetrahydronaphthalen-2-amine **12b**



The concentration of 8-methoxy-1,2,3,4-tetrahydronaphthalen-2-amine was determined with UV detection at 210 nm using a linear gradient 5-50% B over 15 min at 1 mL/min (A = water with 0.1% TFA and B = acetonitrile). The 8-methoxy-1,2,3,4-tetrahydronaphthalen-2-amine eluted at a retention time of 10.3 min. A quantitative NMR study calculated the amine purity as 92.3% therefore yields were adjusted accordingly. Results were verified in duplicate.

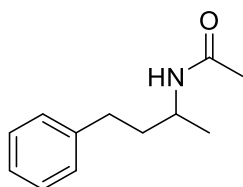


2.2 Chiral HPLC

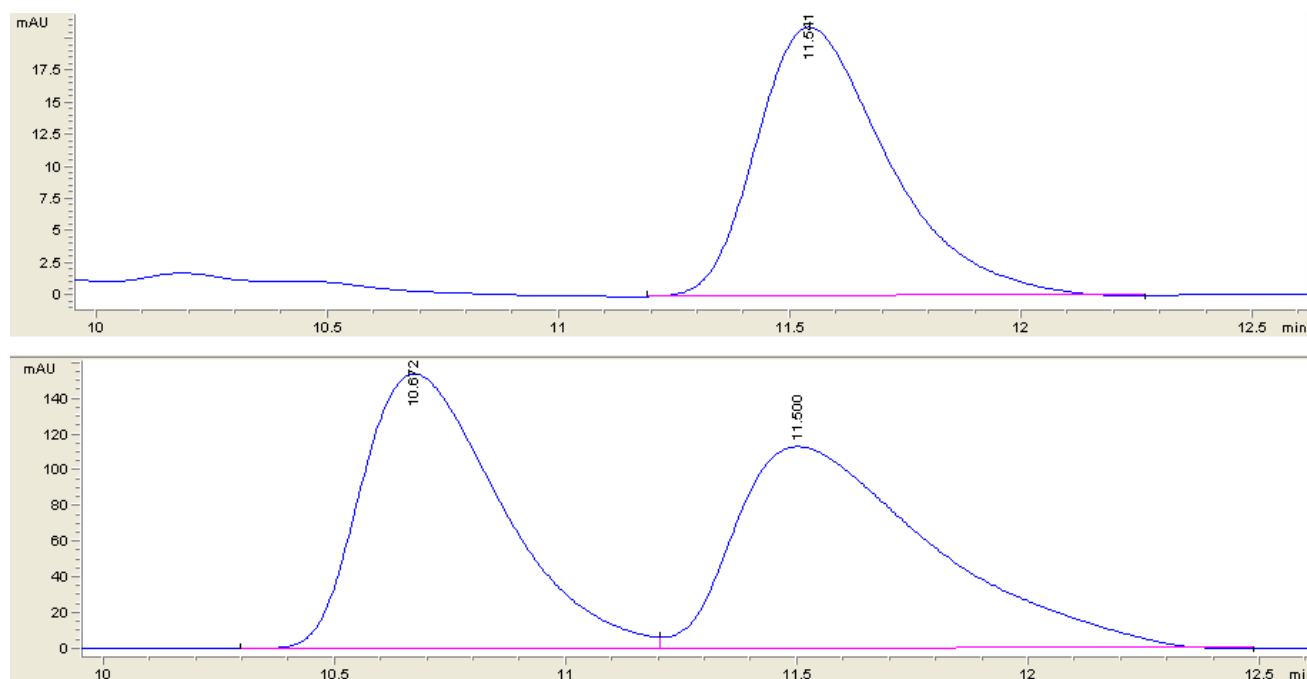
Enantiomeric excesses (e.e.s) of chiral amines **8b** and **12b** were determined by chiral HPLC on an Agilent HP 1100 system using a Chiralpak AD-H column (250 × 4.6 mm) with UV detection at 220 nm using an isocratic mobile phase of 5:95 isopropanol:*n*-hexane over 30 min at 1 mL/min.

Mechanochemical reactions were carried out as described above. 1 mL of the reaction mixture after rinses (10 mM) was extracted with dichloromethane (1 mL). The product was then derivatised using acetic anhydride (1.9 μL) overnight, concentrated to dryness, redissolved in 1:9 IPA:*n*-hexane and analysed by HPLC.

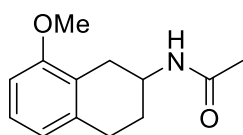
N-(4-Phenylbutan-2-yl)acetamide (derivatised **8b**)



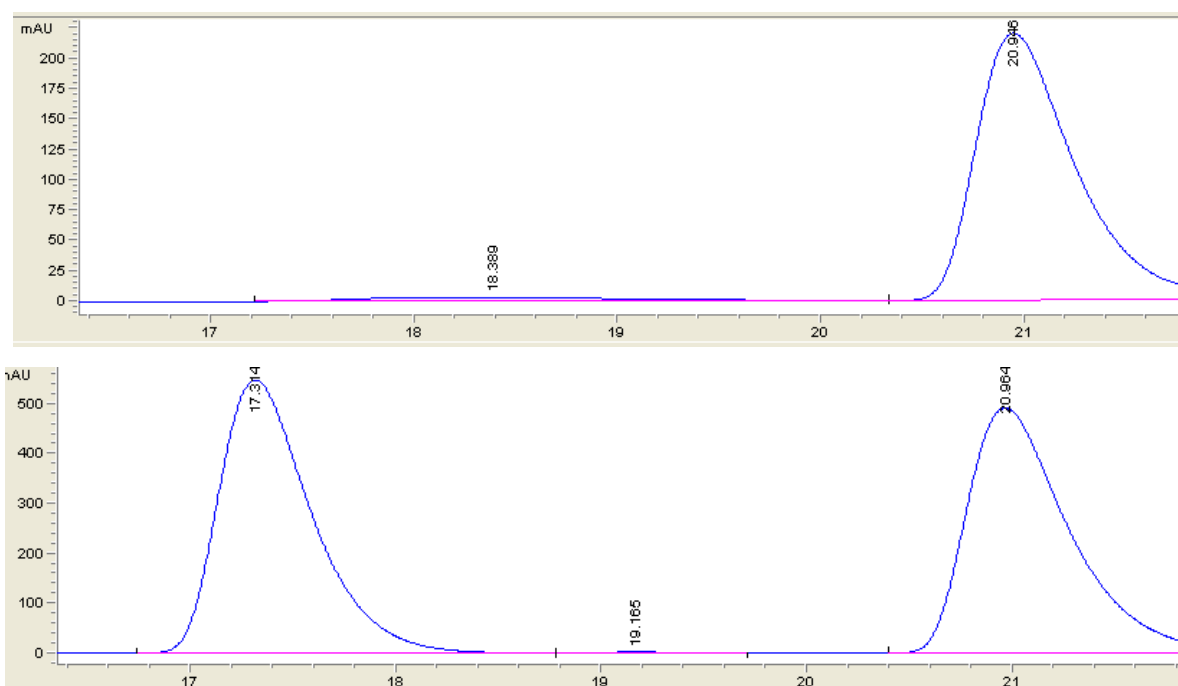
N-(4-phenylbutan-2-yl)acetamide (from Alfa Aesar) eluted at a retention time of 10.7 and 11.5 min for the racemic standard, and only 11.5 min for the enzymatic reaction. Comparison to the literature confirmed the product as the (2*S*)-isomer.³



N-(8-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (derivatised **12b**)



N-(8-Methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)acetamide (synthesised as below) eluted at a retention time of 17.3 and 21.0 min for the racemic standard, and only 21.0 min for the enzymatic reaction. It has been reported that *Cv*-TAm forms (*S*)-**12b**.⁴

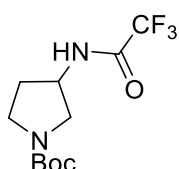


2.3 Chiral GC

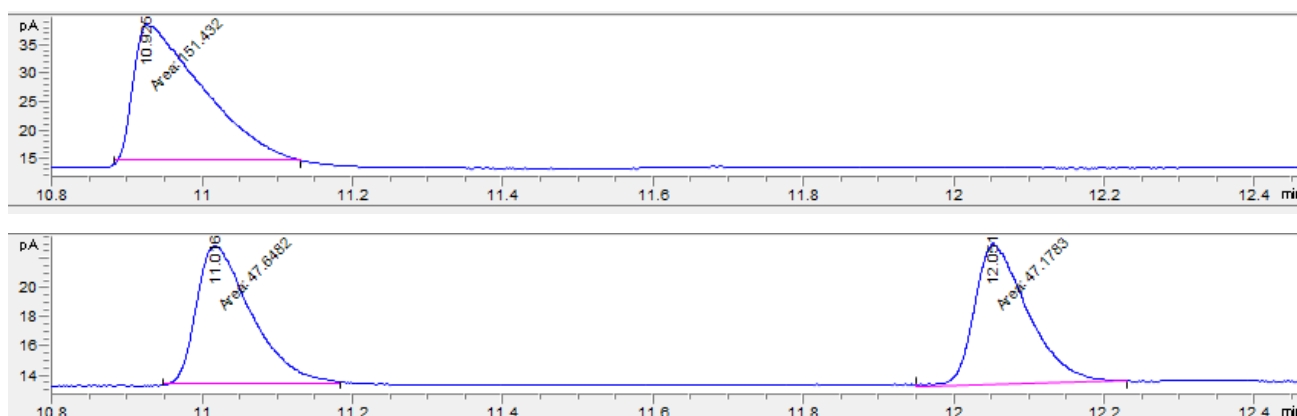
The e.e.s of chiral amines **10b** and **11b** were determined by GC analysis using an Agilent 7820A GC System with a Supelco Beta Dex 225 capillary GC column 30 m × 250 μm × 0.25 μm with an initial temperature of 150 °C, 2 min hold, ramp 3 °C/min to 220 °C, flame ionization detector at 300 °C, inlet temperature 250 °C, split ratio 5:1, flow 8 mL/min with hydrogen gas and 1 μL injection volume.

Mechanochemical reactions were carried out as described above. 1 mL of the reaction mixture after rinses (10 mM) was extracted with dichloromethane (1 mL). The product was then derivatised using trifluoroacetic anhydride (14 μL) overnight, concentrated to dryness, redissolved in ethyl acetate and analysed by GC.

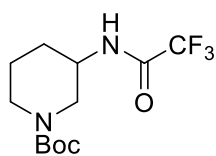
tert-Butyl 3-(2,2,2-trifluoroacetamido)pyrrolidine-1-carboxylate (derivatised **10b**)



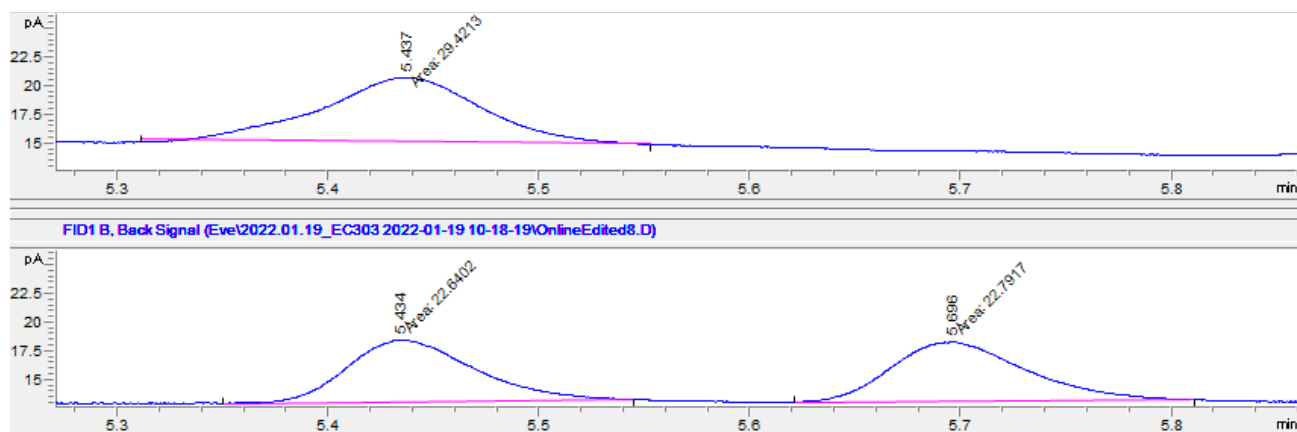
tert-Butyl 3-(2,2,2-trifluoroacetamido)pyrrolidine-1-carboxylate (from Alfa Aesar) eluted at a retention time of 11.0 and 12.0 min for the racemic standard, and only 11.0 min for the enzymatic reaction. By comparison to reported (*S*)-selective TAmS, and the data below for derivatised **11b**, this 11.0 min peak was assigned as the (*S*)-isomer.



tert-Butyl 3-(2,2,2-trifluoroacetamido)piperidine-1-carboxylate (derivatised **11b**)



tert-Butyl 3-(2,2,2-trifluoroacetamido)piperidine-1-carboxylate (from Alfa Aesar) eluted at a retention time of 5.4 and 5.7 min for the racemic standard, and only 5.4 min for the enzymatic reaction. Comparison to the literature confirmed the product as the (*S*)-isomer.⁵



3 Synthetic Methods

3.1 General methods

All reagents were obtained from commercial suppliers and used without further purification. Solvents were evaporated under reduced pressure using a Büchi rotary evaporator. Room temperature is defined as between 18-22 °C.

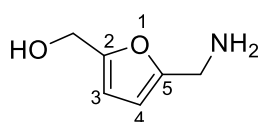
¹H and ¹³C NMR spectra were recorded using Bruker Avance Neo 700 and Bruker Avance III 600. Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to the residual solvent peak: CDCl₃ (δ 7.26 ppm in ¹H and δ 77.2 ppm in ¹³C). Coupling constants (J) are quoted in Hertz (Hz). The ¹H NMR spectra are reported as follows: ppm (number of protons, multiplicity, coupling constants, assignment). NMR assignments use numbering independent from IUPAC, using two-dimensional (COSY, HSQC, HMBC) NMR spectroscopy to assist the assignment.

Low Resolution Mass Spectra (LRMS) were recorded on a Waters LCT Premier Q-TOF or Waters Aquity UPLC-SQD operating in ESI mode, or Thermo MAT900 if EI/CI, or on a Micromass MALDI-TOF depending on the compound.

3.2 Synthesis of amines

Where possible, amines were obtained from commercial suppliers and used without further purification for use in calibration curves. When this was not possible, they were isolated as below.

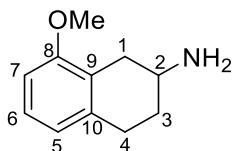
(5-Aminomethyl-furan-2-yl)-methanol **6b**⁶



(5-Aminomethyl-furan-2-yl)-methanol **6b** was isolated from the transaminase reaction (2 mL scale reaction in 5 mL stainless steel jars as described above). The jars were rinsed with water and methanol, and insoluble material was removed by centrifugation (4 °C, 12000 rpm, 30 min). The supernatant was then concentrated to dryness, redissolved in water (10 mL), acidified to below pH 2 with HCl and washed with EtOAc (3 × 10 mL). This was then basified to over pH 10 with NaOH and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried with MgSO₄, filtered, and concentrated to dryness. A quantitative NMR study with dimethyl sulfone calculated the amine purity as 37.5% therefore yields were adjusted accordingly. **¹H NMR** (700 MHz; CDCl₃) δ 6.20 (1H, d, J = 3.1 Hz, 3-H), 6.08 (1H, d, J = 3.1 Hz, 4-H), 4.57 (2H, s, CH₂OH), 3.81 (2H, s, CH₂NH₂), 1.79 (3H, s, NH₂ &

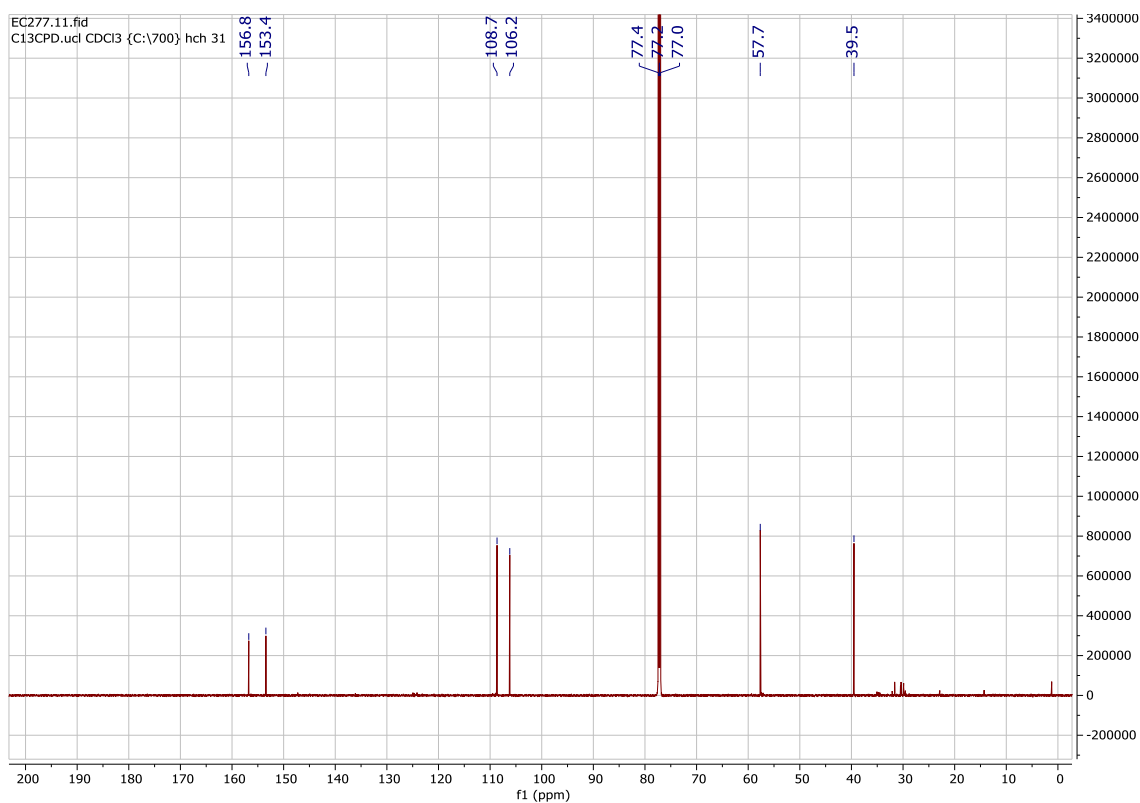
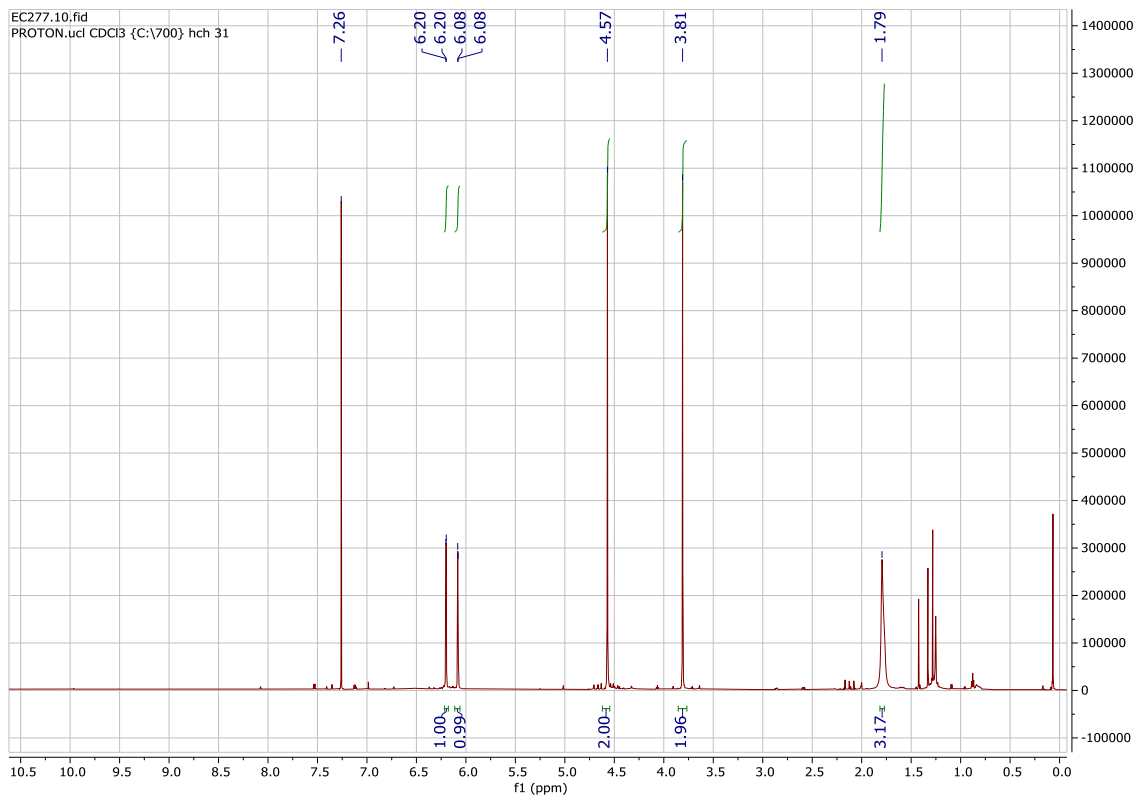
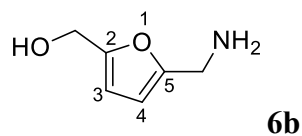
OH); ^{13}C NMR (176 MHz; CDCl_3) δ 156.8, 153.4, 108.7, 106.2, 57.7, 39.5; m/z [ESI+] 127 ($[\text{M}+\text{H}]^+$, 4%), 111 ($[\text{M}-\text{NH}_2]^+$, 100%). All spectral data corresponded to that reported in the literature.⁶

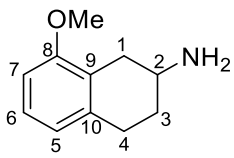
8-Methoxy-1,2,3,4-tetrahydronaphthalen-2-amine **12b**⁷



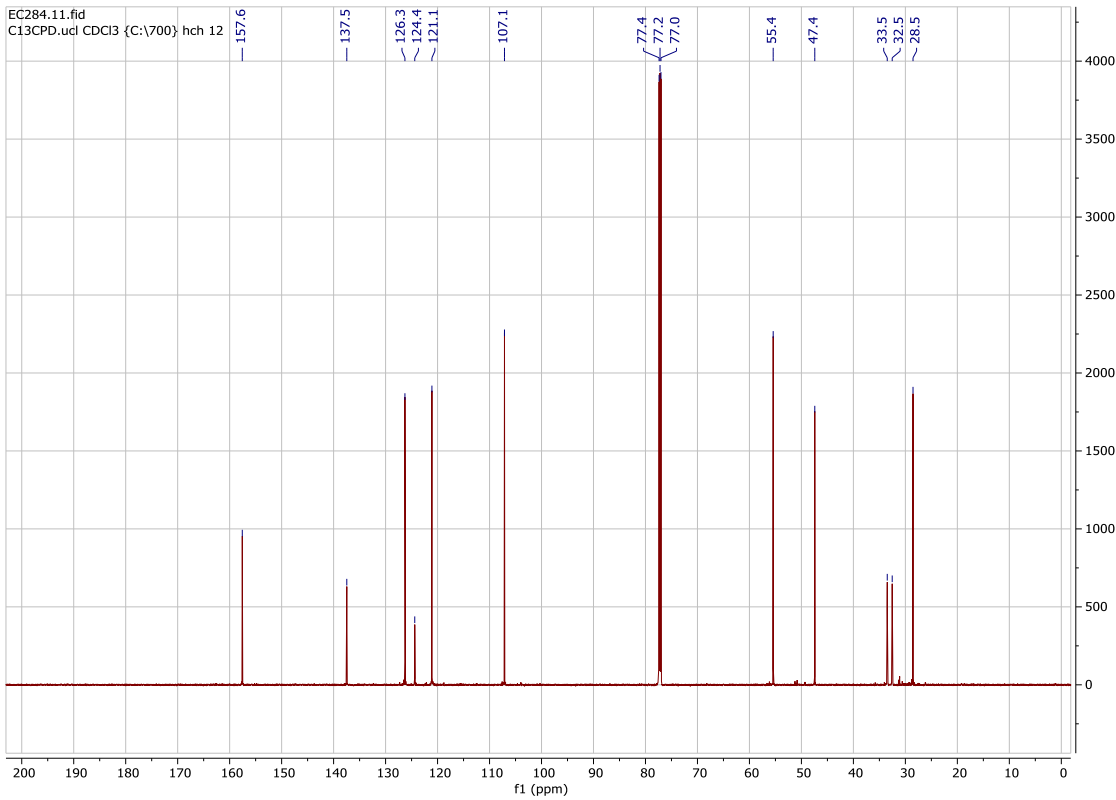
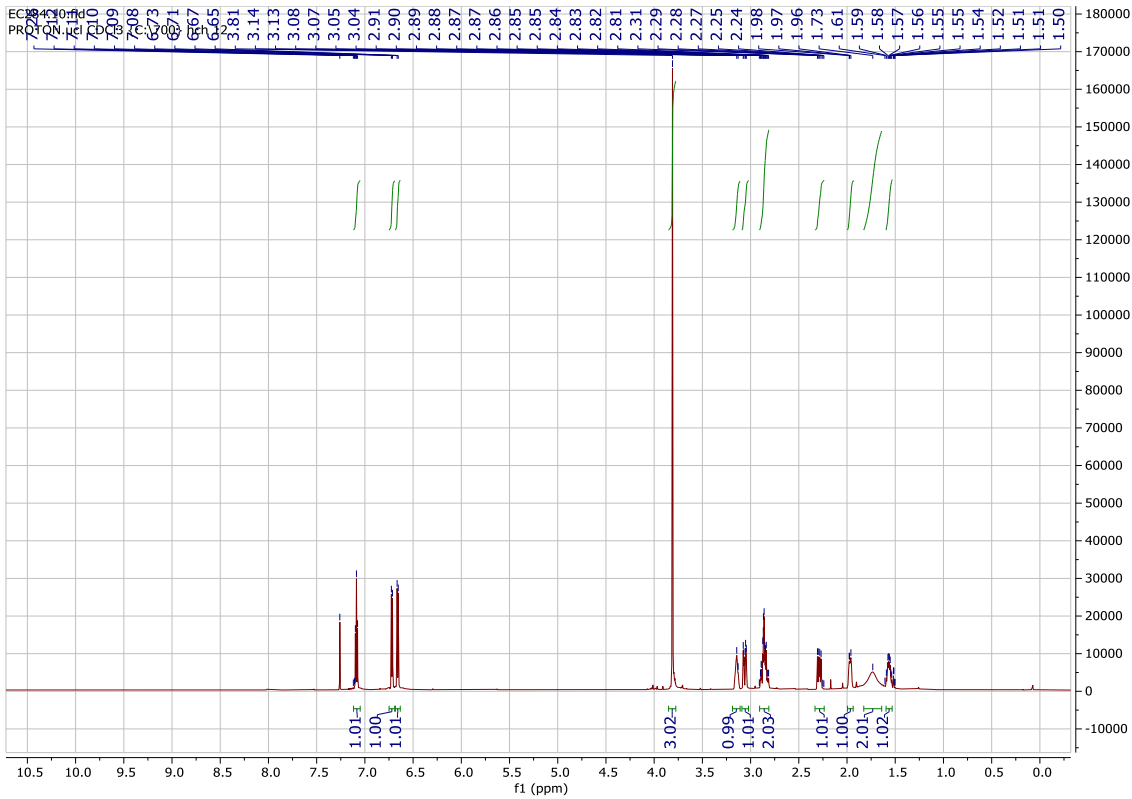
To a solution of 8-methoxy-2-tetralone **12a** (0.44 mL, 2.84 mmol, 1 equiv.) in methanol under argon was added ammonium acetate (2.19 g, 28.4 mmol, 10 equiv.) and sodium cyanoborohydride (125 mg, 1.4 mmol, 1.2 equiv.) and the solution stirred for 48 h. The solvent was removed under reduced pressure then the resulting solid was redissolved in water (30 mL), acidified to below pH 2 with HCl, washed with dichloromethane (2×30 mL), basified to over pH 10 with NaOH then extracted with dichloromethane (2×30 mL). The combined organic layers were dried with MgSO_4 , filtered and concentrated under reduced pressure to yield the product (210 mg, 42%). A quantitative NMR study with 1,4-dioxane calculated the amine purity as 92.9% therefore yields were adjusted accordingly. ^1H NMR (700 MHz; CDCl_3) δ 7.09 (1H, m, 6-H), 6.72 (1H, d, $J = 7.6$ Hz, 5-H), 6.66 (1H, d, $J = 8.1$ Hz, 7-H), 3.81 (3H, s, OMe), 3.14 (1H, m, 2-H), 3.06 (1H, dd, $J = 16.8, 5.1$ Hz, 1-H_H), 2.86 (2H, m, 4-H), 2.29 (1H, dd, $J = 16.8, 9.3$ Hz, 1-H_H), 1.97 (1H, m, 3-H_H), 1.73 (2H, s, NH_2), 1.56 (1H, m, 3-H_H); ^{13}C NMR (176 MHz; CDCl_3) δ 157.6, 137.5, 126.3, 124.4, 121.1, 107.1, 55.4, 47.4, 33.5, 32.5, 28.5; m/z [ESI+] 178 ($[\text{M}+\text{H}]^+$, 100%), 161 ($[\text{M}-\text{NH}_2]^+$, 27%). All spectral data corresponded to that reported in the literature.⁶

4 NMR spectra





12b



5 References

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