

## **Supporting information**

### **Synthesis of Protected 3-Aminopiperidine and 3-Aminoazepane Derivatives Using Enzyme Cascades**

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## **Materials and methods**

All commercial reagents and solvents used in this work were commercially purchased from Sigma-Aldrich (Poole, Dorset, UK), Alfa-Aesar (Heysham, Lancashire, UK), Acros Organics (Loughborough, UK), Santa Cruz Biotechnology (Dallas, Texas, U.S.A.) or Fluorochem (Hadfield, Derbyshire, UK) and used without further purification.

A Bruker Avance 400 spectrometer was used to record NMR spectra with chemical shifts reported in ppm relative to tetramethylsilane (TMS). Coupling constants (J) are reported in Hz.

High-resolution mass spectrometry (HRMS) was recorded using a Waters LCT time-of-flight mass spectrometer. GC-MS analysis was performed on an Agilent 7890B GC instrument fitted with an auto sampler and an Agilent HP-1 column and an Agilent 5977 MSD detector for low-resolution mass spectrometry. GC-FID analysis was performed on an Agilent 6850 GC fitted with a flame ionisation detector (FID), auto sampler and HP-1 column (30 m x 0.25 mm ID, 0.25  $\mu$ m film thickness) from Agilent Technologies (Santa Clara, CA, USA).

Normal-phase HPLC was carried out on an Agilent 1100 series system (USA) equipped with a G1379A degasser, G1313A binary pump, G1367A well plate auto sampler, G1316A temperature-controlled compartment and a diode array detector. For chiral analysis a Daicel (Osaka, Japan) CHIRALPAK® IC column (particle size 5  $\mu$ m, dimensions: 4.6 x 250 mm) was used: 4.6 mm diameter and 250 mm length. Injection volumes of 10  $\mu$ L were used and the DAD detector recorded absorbance at 265 nm.

Reverse Phase HPLC was carried out on an Agilent 1260 Infinity II Series system equipped with a G1379A degasser, G1312A binary pump, G1367A well plate auto sampler, G1316A temperature controlled compartment and a diode array detector. A Kinetex® 5 $\mu$ m, C18 100 Å, 50 x 2.1 mm column was used as a stationary phase.

## Biocatalyst Production

Chemically competent *E. coli* cells were transformed with DNA plasmid vectors that contain genes for the desired protein (biocatalyst) and grown on LB agar plates containing 30 mg mL<sup>-1</sup> antibiotic (kanamycin) as shown in **Table S1**.

**Table S1:** Biocatalysts vectors and cell strains used in this work. BL – 21 cells were purchased from New England Biolabs and BL – 21\* cells were purchased from Qiagen.

Protein	Organism	Plasmid	Cell Strain
GOase M <sub>3-5</sub>	<i>Fusarium graminearum</i>	pET30	BL – 21*
GOase F <sub>2</sub>	<i>Fusarium graminearum</i>	pET30	BL – 21*
AdRedAm	<i>Ajellomyces Dermatitidis</i>	pET28a	BL – 21
IR – 23	<i>Cystobacter ferrugineus</i>	pET28a	BL – 21
IR – 49	<i>Mycobacterium magaritense</i>	pET28a	BL – 21
IR – 102	<i>Unknown</i>	pET28a	BL – 21
IR – 110	<i>Unkown</i>	pET28a	BL – 21

## Galactose oxidase (GOase) production

A single colony harbouring a GOase gene was selected and used to inoculate 5 mL of lysogny broth (LB) medium containing the appropriate amount of antibiotic in a 15 mL falcon tube and incubated overnight at 37 °C shaking at 250 rpm. 500 µL of the preculture was used to inoculate a 250 mL of auto induction medium (8ZY-4LAC)<sup>1</sup> supplemented with 250 µL of kanamycin (30 mg mL<sup>-1</sup>) in a 2 L baffled flask and further incubated at 250 rpm at 26 °C for 60 h. The cell cultures were harvested by centrifugation at 4000 rpm for 30 min at 4 °C and the supernatant was discarded. The cell pellets were washed with NaPi buffer and pelleted again by the same method. The wet cell pellets were stored at -20 °C.

To purify the protein the thawed cell pellets were first resuspended in 15 – 20 mL of TritonX-100 lysis buffer<sup>2</sup> and incubated at 4 °C for 20 min with gradual shaking. The suspension was then lysed by ultra-sonification (20 sec ON, 20 sec OFF, 20 sec intervals) while submerged in an ice bath. The lysate was clarified by centrifugation at 20,000 rpm for 40 min at 4 °C. The clarified supernatant was dialysed overnight against Buffer NP (50 mM NaPi buffer, 300 mM NaCl, pH 8) at 4 °C. The supernatant was then loaded onto a Strep-TagII® column which had been equilibrated with 50 mL NP Buffer and the column was subsequently washed with 30 mL NP buffer to remove any non-tagged proteins. The GOase was eluted with 40 mL NPD buffer

(50 mM Na<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 5 mM desthiobiotin, pH 8) and concentrated by centrifugation in vivaspin columns. The protein was dialysed overnight at 4 °C against 50 mM NaPi (pH 7.4) supplemented with copper sulphate. After, the protein was dialysed again at 4 °C against NaPi buffer to remove excess Cu. The protein concentration was then determined using a NanoDrop™ spectrometer (typically 3 – 5 mg ml<sup>-1</sup>). The samples were divided into 1.5 mL Eppendorf tubes, flash-frozen and then stored at -80 °C until they were used in the biotransformation reactions.

### **Imine Reductase (IRED) production**

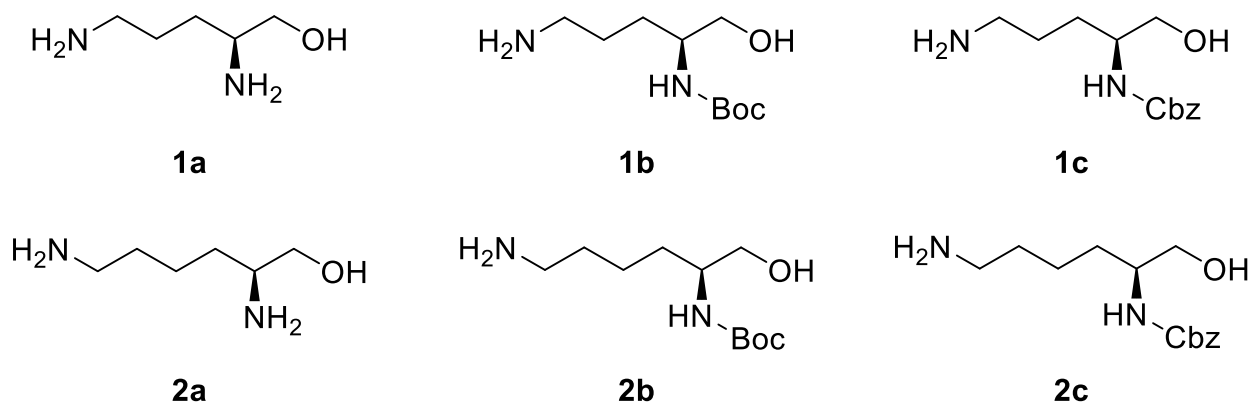
A single colony harbouring an IRED gene was added to 20 mL LB medium supplemented with 20 µL kanamycin (30 mg ml<sup>-1</sup>) and incubated overnight at 250 rpm and 30 °C. The full volume of this preculture was used to inoculate a 400 mL of TB media, supplemented with 400 µL of kanamycin, in a 2 L baffled flask and further incubated at 37 °C, 250 rpm until optical cell density (OD<sub>600</sub>) of 0.6 was reached. At this point, protein expression was induced by inoculating the flask with 400 µL of isopropyl-beta-D-1-thiogalactopyranoside (IPTG, 0.1 M). The flask was then incubated at 22 °C at 200 rpm for 20 h. The cells were harvested by centrifugation at 4000 rpm for 30 min at 4 °C. The cell pellets were washed with reaction buffer and centrifuged again using the same conditions and the supernatant discarded. The cell pellets were stored at -20 °C in 50 mL falcon tubes until further use.

For purification, the thawed cell pellets were resuspended in 4 – 5 mL (per g of cell pellet) in 10 % Buffer B (100 mM NaPi buffer, 300 mM NaCl, 30 mM imidazole, pH 7.5) and lysed by ultra-sonication (60 sec ON, 120 sec OFF, 40 AMP, 4 cycles) while samples were submerged in an ice bath. The lysed cells were clarified by ultra-centrifugation at 18,000 rpm at 4 °C for 60 min. The clarified supernatant was filtered through a cellulose membrane (0.45 µm) and loaded onto a His-Trap Crude FF column (GE Healthcare) charged with 0.1 M nickel sulphate equilibrated with 10% buffer B. The column was washed with 15 mL 10% buffer B and then 15 mL 20% buffer B. The His-tagged IRED protein was then eluted with 100% buffer B and 1 – 2 mL fractions were collected. The protein concentration of each fraction was monitored by a thermofisher NanoDrop™ microvolume spectrophotometer. Fractions containing pure protein were combined and then concentrated using a membrane 30,000 MWCO PES centrifugal concentrator (Sartorius, UK) to remove excess imidazole. Buffer A (100 mM NaPi buffer, 300 mM NaCl, pH 7.5) was added to the samples until desired concentration was achieved. The pure protein was divided into 1 mL aliquots, flash frozen and stored at – 80 °C until further use.

# Biotransformation Methods

## Substrate scope

A panel of 6 amino alcohols were derived from ornithine and lysine ("Chemical synthesis") and tested in the proposed GOase – IRED cascade (**Figure S1**).



**Figure S1:** Amino alcohol substrate panel

## Analytical scale biotransformations

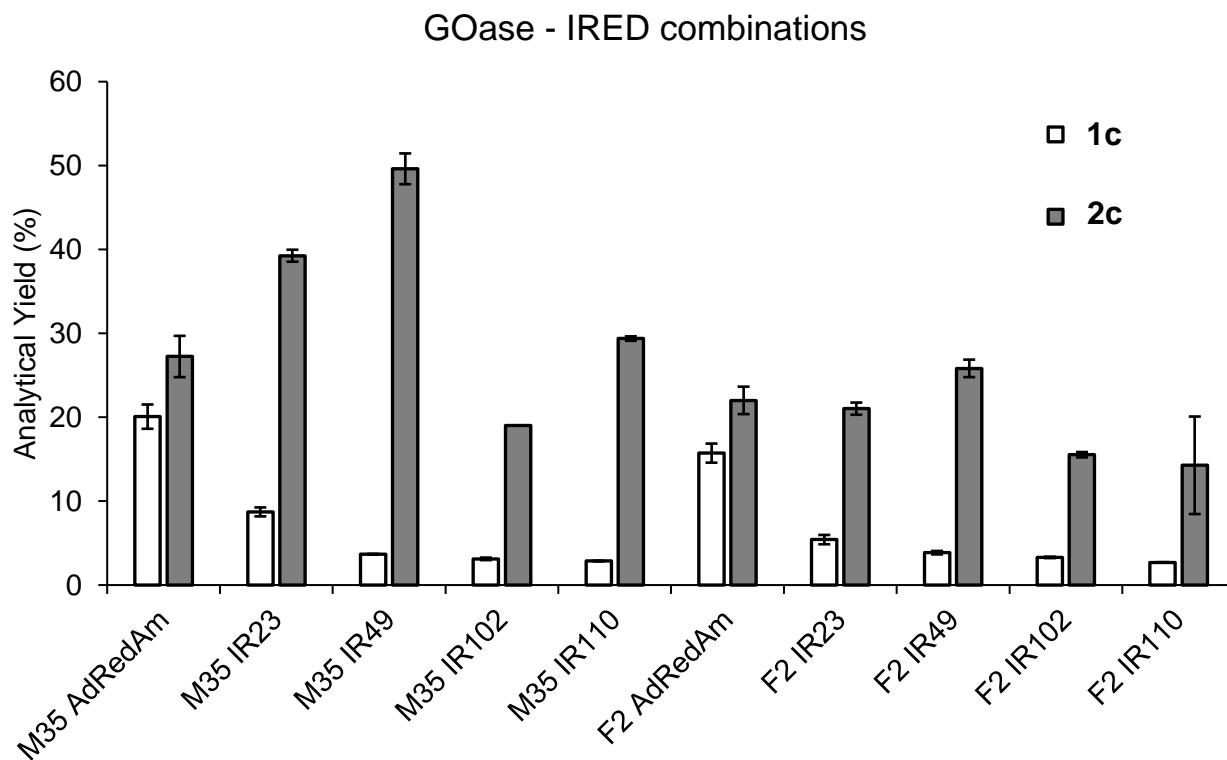
Triplicate reactions were carried out in 2 mL Eppendorf tubes with a total reaction volume of 500  $\mu$ L. Each reaction contained components diluted from stock solutions, with final concentrations of 3 mM amino alcohol substrate (**1a – c** or **2a – c**), 1 mg ml<sup>-1</sup> IRED, 0.25 mg ml<sup>-1</sup> GOase, 0.08 mg ml<sup>-1</sup> catalase, 0.064 mg ml<sup>-1</sup> HRP, 0.5 mM NADP<sup>+</sup>, 0.2 mg ml<sup>-1</sup> GDH lysate (CDX-901) and 25 mM glucose in 100mM NaPi pH 7.5. Reactions were incubated at 30 °C, 200 rpm for 24 h.

After incubation, the biotransformations that contained substrates **1a** and **2a** were quenched with 500  $\mu$ L MeOH and the precipitate was removed by filtration. Derivatisation using *p*-toluenesulfonyl chloride (PTSC) was then carried out as previously reported.<sup>3</sup> 150  $\mu$ L of PhEtOH in MeOH (30 mM), 800  $\mu$ L PTSC 8mM in MeOH and 50  $\mu$ L MeOH (1% TEA) was added and then sonicated for 5 min. The samples were filtered again and analysed on reverse-phase HPLC for qualitative assessment.

After incubation, the biotransformations that contained substrates **1b**, **1c**, **2b** and **2c** were basified with 20  $\mu$ L of 10 M NaOH and extracted into 500  $\mu$ L DCM (containing 5 mM PhEtOH). The organic extract was dried over anhydrous MgSO<sub>4</sub> and analysed by GC-FID. Analytical yield of products were determined by comparison with a calibration curve made from commercial product standards and 5 mM PhEtOH (internal standard). No product formation was detected for the Boc-protected substrates (**1b** and **2b**) whereas product was detected for the biotransformations that used the Cbz-protected substrates (**1c** and **2c**).

## Initial screening of proposed GOase – IRED cascade.

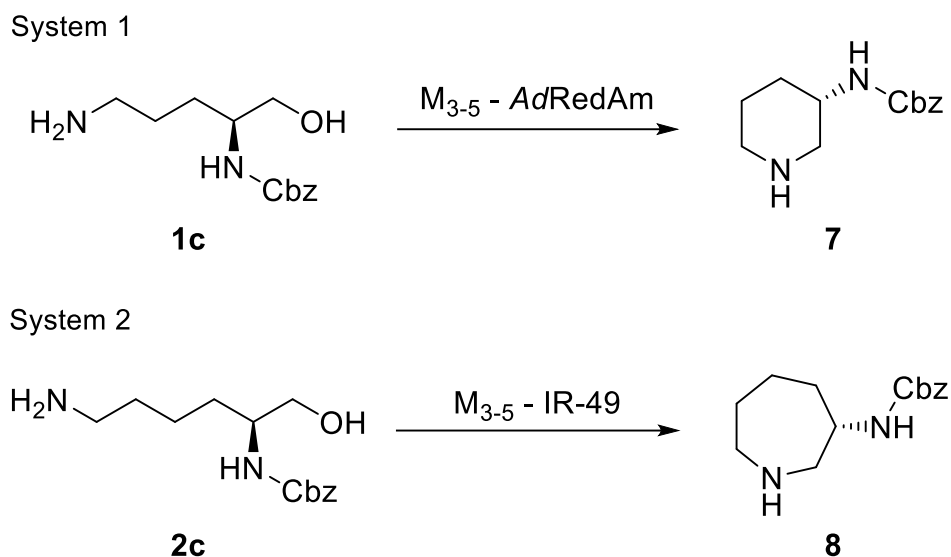
Preliminary tests using substrates **1a**, **2a**, **1b** and **2b** with each possible combination of GOase and IRED homologs showed there was no major product formation. Whereas, screening results for **1c** and **2c** are shown in **Figure S2**.



**Figure S2:** Analytical yield of cascade products determined from GC-FID using substrates **1c** and **2c**.

## Analytical scale biotransformation optimisations

The reaction of **1c** to **7** was optimised using GOase M<sub>3-5</sub> and *AdRedAm* and defined as System 1. Whereas, the reaction of **2c** to **8** was optimised using GOase M<sub>3-5</sub> and IR-49 and defined as System 2 (**Figure S3**). Reaction optimisation biotransformations were carried out as described above (**Analytical scale biotransformations**), with each reaction component being investigated individually. GOase and IRED concentrations of 0.25 mg ml<sup>-1</sup>, 0.5 mg ml<sup>-1</sup>, 0.75 mg ml<sup>-1</sup> and 1 mg ml<sup>-1</sup> were tested as well as substrate concentrations of 1 mM, 2 mM, 3 mM and 5 mM. Reactions were carried out at 20 °C, 25 °C, 30 °C and 37 °C as well as buffer pH 6, 7, 7.5 and 8. It was found that there was a large variation in analytical yield of products **7** and **8** compared to the yields determined from the initial trials. It was determined that the Cbz-protected products were degraded on the GC column yielding quantities of benzyl alcohol (which was identified by GC-MS analysis).



**Figure S3:** Oxidation of amino alcohols (**1c** and **2c**) by GOase  $M_{3-5}$  followed by subsequent spontaneous cyclisation and imine reduction by AdRedAm (System 1) or IR-49 (System 2), respectively.

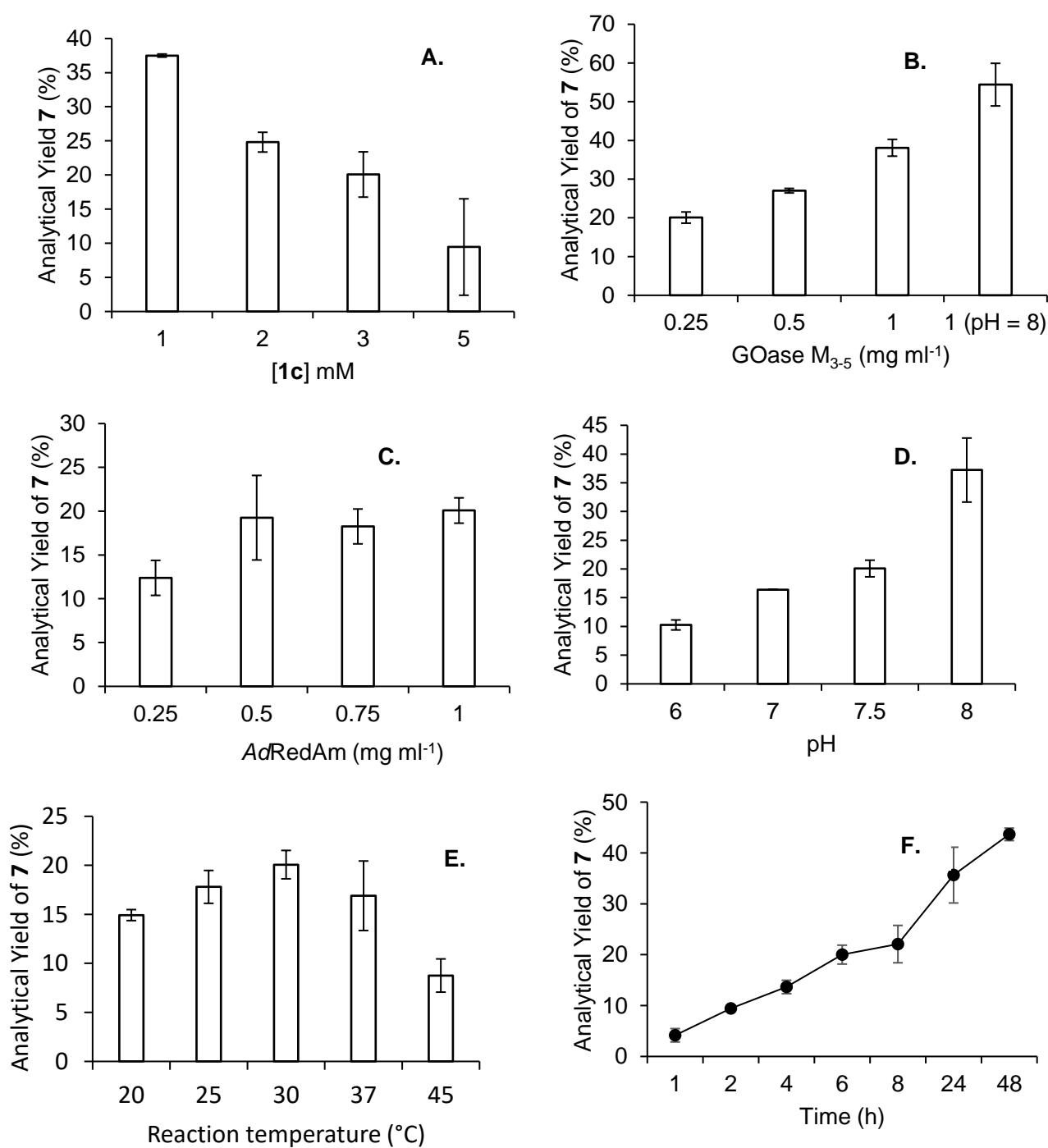
## Time Course

The progression of the enzymatic cascade for System 1 and System 2 were carried out in the same conditions as previously stated but at pH 8. Samples were taken after 1, 2, 4, 6, 8, 24 and 48 h of incubation and monitored by GC-FID. Results of the optimisations and time course experiments are shown below in **Figure S4**.



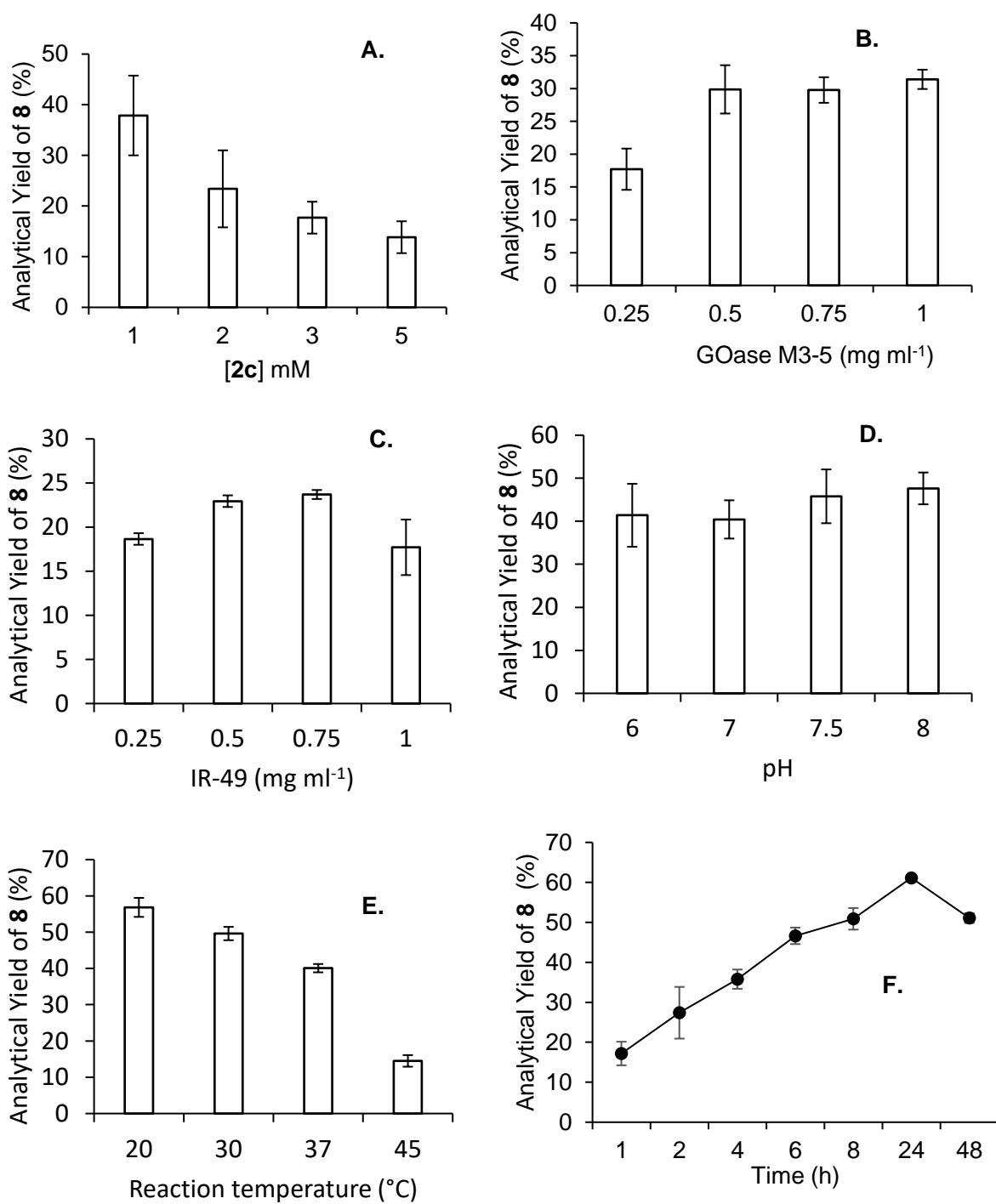
## Optimisation data

System 1



**Figure S4:** Reported analytical yield of 7 from 1c as a function of: **A.** Substrate concentration **B.** GOase M<sub>3-5</sub> concentration **C.** AdRedAm concentration **D.** Reaction pH **E.** Reaction temperature. **F.** Time-course experiment.

## System 2



**Figure S5:** Reported analytical yield of **8** from **2c** as a function of: **A.** Substrate concentration **B.** GOase M3-5 concentration **C.** IR-49 concentration **D.** Reaction pH **E.** Reaction temperature. **F.** Time-course experiment.

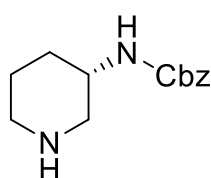
## Preparative scale biotransformations

Following optimisation, reactions for System 1 and System 2 were scaled up. Both scale ups were carried out in a 1 L Erlenmeyer flask with a total reaction volume of 90 mL. Reactions were incubated in an orbital shaker at 30 °C at 200 rpm and product formation was followed by taking 500  $\mu$ L aliquots, extracting them following the previously described analytical scale protocol and analysing them on GC-FID. The reaction was basified with 4 mL of 10 M NaOH and extracted into DCM (3 x 100 mL). The emulsion formed was filtered through a pad of Celite® and washed with DCM. The organic layer was concentrated under vacuum. The crude product was dissolved in EtOAc (30 mL) and washed with HCl (1 M, 10 mL). The aqueous layer was then basified to pH 10, extracted with  $\text{CHCl}_3$  (3 x 20 mL), dried over anhydrous  $\text{MgSO}_4$  and concentrated under vacuum yielding product. Both products **7** and **8** were identified by NMR and HRMS and compared to the product standards.

**Table S2:** Scaled-up biotransformations contained final concentrations of 3 mM substrate, 0.08  $\text{mg mL}^{-1}$  catalase, 0.064  $\text{mg mL}^{-1}$  HRP, 0.5 mM  $\text{NADP}^+$ , 0.2  $\text{mg mL}^{-1}$  GDH lysate, 25 mM glucose in 100 mM NaPi (pH 8).

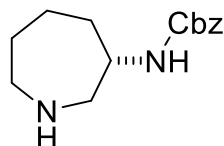
Substrate	$\text{M}_{3-5}$ ( $\text{mg mL}^{-1}$ )	IR-49 <sup>l</sup> ( $\text{mg mL}^{-1}$ )	AdRedAm ( $\text{mg mL}^{-1}$ )	Isolated product yield (%)
<b>1c</b>	1	0.25	/	<b>7</b> (16 %)
<b>2c</b>	0.5	/	0.25	<b>8</b> (54 %)

### (S)-Benzyl piperidin-3-ylcarbamate (**7**)



Scale-up yielded a yellow oil (10 mg, 16 %).  **$^1\text{H NMR}$**  (400 MHz, Chloroform-*d*)  $\delta$  = 7.38 – 7.28 (m, 5H), 5.29 (s, 0H), 5.09 (s, 2H), 3.66 (s, 1H), 3.05 (dd,  $J$ =11.8, 3.4, 1H), 2.80 (ddd,  $J$ =12.5, 6.5, 3.0, 1H), 2.69 (dt,  $J$ =12.8, 6.1, 1H), 2.57 (dd,  $J$ =11.8, 7.1, 1H), 1.82 (td,  $J$ =8.5, 4.0, 1H), 1.66 (s, 3H), 1.48 (ddt,  $J$ =11.2, 7.5, 3.3, 2H), 1.27 (d,  $J$ =11.6, 1H).  **$^{13}\text{C NMR}$**  (101 MHz, Chloroform-*d*)  $\delta$  = 155.65, 136.63, 128.51, 128.10, 128.07, 66.55, 65.28, 53.44, 51.82, 47.49, 46.41, 30.69, 29.71, 24.05. **HRMS**  $\text{C}_{13}\text{H}_{18}\text{N}_2\text{O}_2$  calculated  $[\text{M}+\text{H}]^+$  235.144076 found: 235.1441.

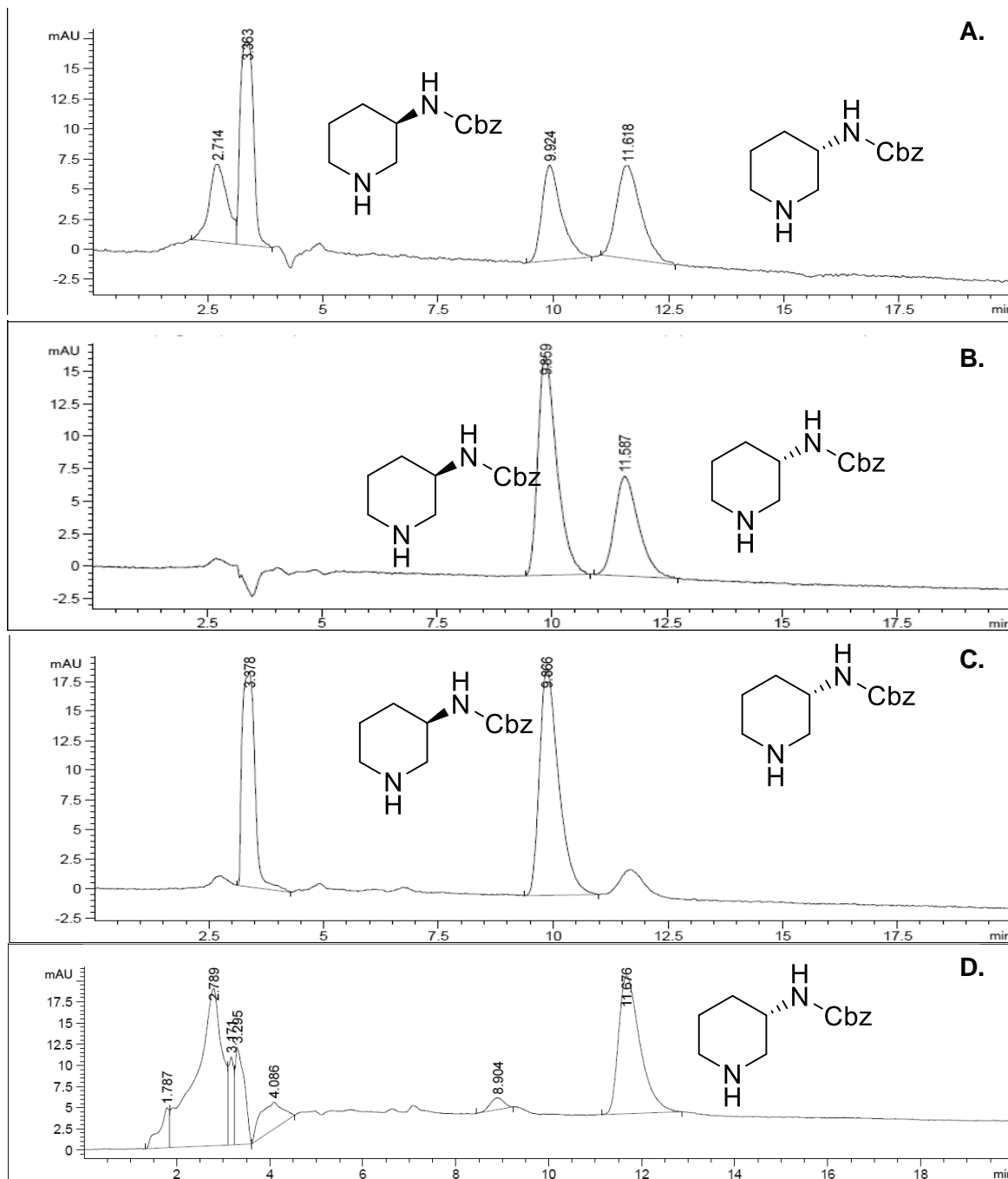
### (S)-Benzyl azepan-3-ylcarbamate (**8**)



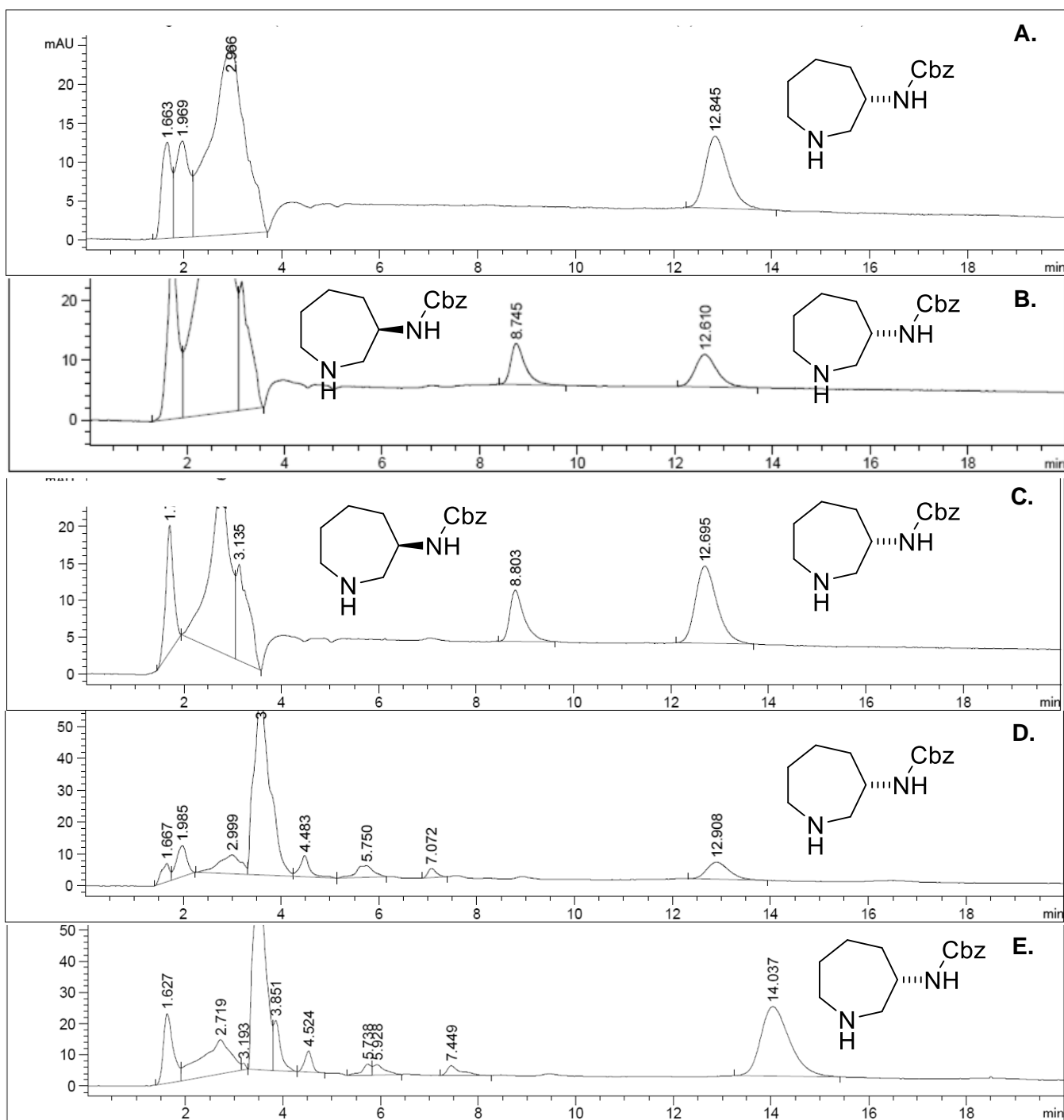
Scale-up yielded a yellow oil (36 mg, 54 %).  **$^1\text{H NMR}$**  (400 MHz, Chloroform-*d*)  $\delta$  = 7.41 – 7.27 (m, 5H), 5.52 (d,  $J$ =8.5, 1H), 5.08 (s, 2H), 3.82 (p,  $J$ =4.2, 1H), 3.01 – 2.72 (m, 3H), 1.78 (td,  $J$ =15.7, 13.7, 6.6, 1H), 1.73 – 1.58 (m, 3H), 1.56 – 1.38 (m, 1H).  **$^{13}\text{C NMR}$**  (101 MHz, Chloroform-*d*)  $\delta$  = 155.63, 136.73, 128.50, 128.11, 128.03, 66.46, 52.97, 50.96, 49.10, 34.27, 29.87, 21.88. **HRMS**  $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_2$   $[\text{M}+\text{H}]^+$  calculated 249.159776. found: 249.1614.

## Determination of enantiomeric excess

The stereochemistry of products **7** and **8** from the scale up GOase-IREC cascades are shown in **Figure S6** and **Figure S7**. The chirality of the starting materials were preserved throughout the enzymatic cascade.



**Figure S6:** Normal phase HPLC trace for compound **7**. **A.** 5 mM racemic authentic standard. **B.** 5 mM racemic standard spiked with (R) authentic standard. **C.** 5 mM (R) authentic standard. **D.** Isolated (S) product from preparative scale biotransformation.



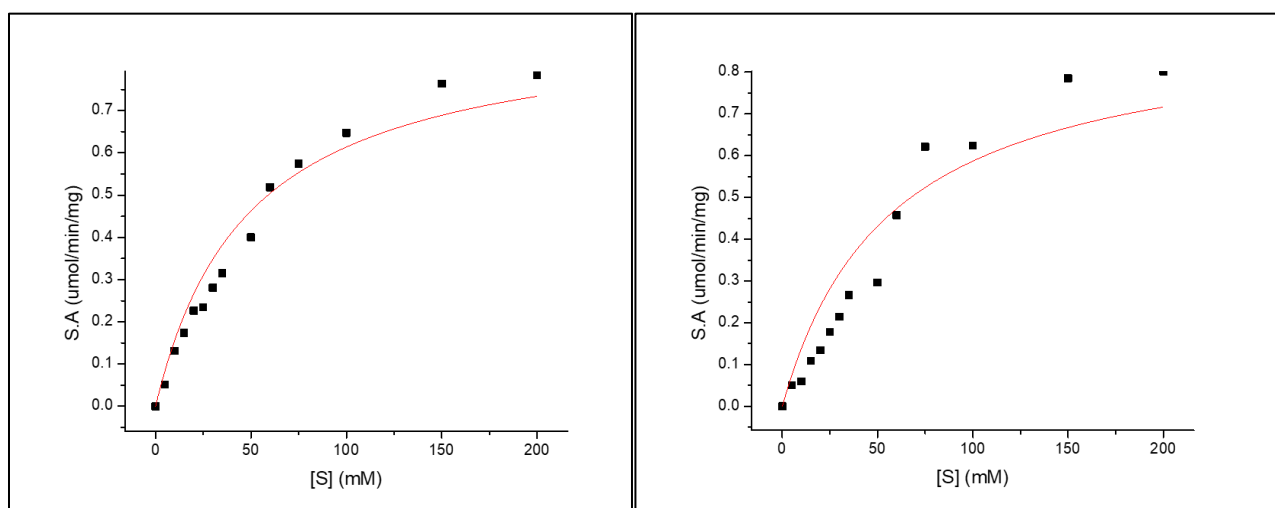
**Figure S7:** Normal HPLC trace for compound 8. **A.** 5mM (S) authentic standard. **B.** 5mM racemic synthesized standard. **C.** Racemic spiked with L-enantiomer. **D.** Isolated (S) from preparative scale biotransformation. **E.** Preparative scale sample spiked with authentic L-enantiomer.

## HRP-ABTS assay and kinetics

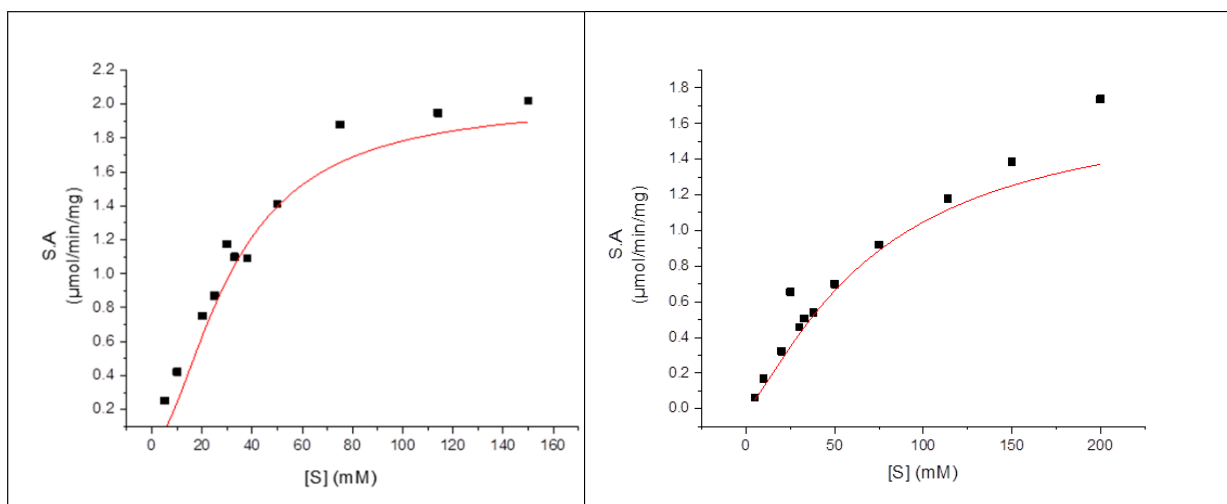
Specific activities of purified GOase F<sub>2</sub> and M<sub>3-5</sub> were measured against substrates **1a – c** and **2a – c** using the previously reported HRP-ABTS assay.<sup>4</sup> In a 96-well plate, purified GOase F<sub>2</sub> and M<sub>3-5</sub> (10  $\mu$ L of 0.43 and 0.48 mg mL<sup>-1</sup> respectively) was diluted with NaPi buffer (100 mM, pH 7.4) that contained ABTS (0.4 mg mL<sup>-1</sup>) and HRP (0.23 mg mL<sup>-1</sup>) to a final volume of 100  $\mu$ L. To initiate the assay reaction 100  $\mu$ L of amino alcohol substrate was added to give a final concentration of 5 mM. The formation of reduced ABTS was recorded for 10 min at 30 °C (420 nm wavelength) using a Tecan 200 plate reader. Each measurement was made in triplicate. Kinetic data of GOase M<sub>3-5</sub> and F<sub>2</sub> against substrates **1c** and **2c** were then determined using the same assay with substrate concentrations ranging from 0 - 250 mM. Kinetic parameters ( $K_M$ ,  $V_{max}$  and  $k_{cat}$ ) were determined using Origin Pro.

Table S3: Measured specific activities of a panel of amino-alcohols comparing GOase M<sub>3-5</sub> and F<sub>2</sub>.

Substrate	Specific Activity ( $\mu\text{mol min}^{-1} \text{mg}^{-1}$ )	
	M <sub>3-5</sub>	F <sub>2</sub>
1a	<0.001	<0.001
1b	<0.001	<0.001
1c	0.051 ( $\pm$ 0.009)	0.05 ( $\pm$ 0.02)
2a	<0.001	<0.001
2b	<0.001	<0.001
2c	0.25 (0.015)	0.062 (0.022)



**Figure S8:** Kinetic study of GOase M<sub>3-5</sub> and F<sub>2</sub> against **1c**.



**Figure S9:** Kinetic study of GOase M<sub>3.5</sub> and F<sub>2</sub> against **2c**.

## Docking

Docking was performed using VINA using default parameters<sup>5</sup> The setup was done with the YASARA molecular modelling program.<sup>6</sup> GOase E1 variant crystal structure (PDB 2WQ8)<sup>1</sup> was used to generate receptors for simulations. The raw crystal structures were first prepared for simulation using the YASARA "Clean" script. The E1 crystal structure was used to model the F2 variant by applying the "Swap" tool as implemented in YASARA, namely the variations Y405F, T406E and I463P have been introduced. The E1 crystal structure was also used to model the M3-5 variant by applying the "Swap" tool as implemented in YASARA, namely the variations K330M and I463P have been introduced. To remove bumps and correct the covalent geometry, the structure was energy-minimised with the NOVA force field<sup>7</sup> using the Particle Mesh Ewald algorithm<sup>8</sup> to treat long range electrostatic interactions. After removal of conformational stress by a short steepest descent minimisation, the procedure continued by simulated annealing (time step 2 fs, atom velocities scaled down by 0.9 every 10th step) until convergence was reached, i.e. the energy improved by less than 0.05 kJ/mol per atom during 200 steps. The simulation was performed in aqueous surrounding removing the therefore added water molecules before starting the docking simulation. L-Cbz-ornithinol **1c** and L-Cbz-lysinol **2c** were considered as ligands in the simulation experiment using their terminal amine functional groups in their protonated form. For the docking simulation, a 15 Å simulation cell around the copper coenzyme was defined for which reasonable functional binding poses were calculated. From the 25 calculated binding poses for each docking run, the catalytically reasonable pose having the highest calculated docking score (strongest binding) was selected based on the requirement to position the hydroxy group that is to be oxidised at the open copper binding site resembling the binding of a co-crystallized acetate in the 2WQ8 crystal structure. The created receptor-ligand complex structures were further processed using the PyMOL software from Schrodinger LLC involving the identification of polar contacts between the ligand and the receptor as well as determination of oxygen-copper distances.

# Chemical Synthesis

## Synthesis of substrates used in biotransformations

Amino alcohols that were used as substrates for enzymatic cascades were synthesised from doubly protected amino acids using the general procedures described below.

### General procedure for carboxylic acid reduction for synthesis of amino alcohol substrates (step i)

To a stirring solution of Z-Orn(Boc)-OH, Boc-Orn(Z)-OH, Z-Lys(Boc)-OH or Boc-Lys(Z)-OH (4.1 mmol) in dry THF (20 mL) under nitrogen at room temperature, triethylamine (570  $\mu$ L, 1 eq.) was added. The solution was then cooled to 0 °C and ethyl chloroformate (390  $\mu$ L) was added dropwise, forming a white precipitate, and stirred for 30 min. NaBH<sub>4</sub> (464 mg, 3 eq) and MeOH (45 mL, slow addition) were added consecutively at 0 °C. The reaction mixture was stirred for an hour at room temperature and concentrated under reduced pressure. The residue was dissolved in a mixture of H<sub>2</sub>O/EtOAc (50 mL, 1:1), separated and the organic phase was washed with brine (30 mL), dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to yield a colourless gel. The crude product was purified by flash chromatography (DCM/MeOH:98-2) to yield compound Z-Orn(Boc)-OI, Boc-Orn(Z)-OI, Z-Lys(Boc)-OI or Boc-Lys(Z)-OI.

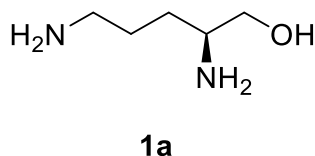
### General procedure for Boc-deprotection for synthesis of amino alcohol substrates (step ii)

Z-Orn(Boc)-OI or Z-Lys(Boc)-OI (1 g, 2.8 mmol) were suspended in a stirring solution of HCl in Et<sub>2</sub>O (2 M, 30 mL) at room temperature, followed by the addition of DCM (15 mL). The reaction mixture was stirred overnight and filtered on filter paper under vacuum. The resultant white solid was washed with Et<sub>2</sub>O (2 x 10 mL) and dried for a further hour, to yield Z-Orn-OI or Z-Lys-OI.

### General procedure for Cbz-deprotection for synthesis of amino alcohol substrates (step iii)

Boc-Orn(Z)-OI or Boc-Lys(Z)-OI (3.4 mmol) were dissolved in dry MeOH (25 mL) under nitrogen. Pd/C (100 mg) catalyst was added to the reaction and the flask was purged and then pressurised with H<sub>2</sub> gas. The solution was stirred overnight and the product was then filtered through a Celite® pad and washed with MeOH (3 x 30 mL). The product was concentrated under reduced pressure to yield Boc-Orn-OI or Boc-Lys-OI.

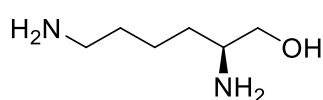
Compound **1a** – (S)-2,5-diaminopentan-1-ol



Synthesised from Boc-Orn(Boc)-OH using step (i) and (ii) consecutively. A brown solid (510 mg, 35 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>)  $\delta$  = 3.83 – 3.57 (m, 2H), 3.33 – 3.23 (m, 1H), 2.99 (t, J=7.0, 2H), 1.86 - 1.64 (m, 4H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>)  $\delta$  = 60.31, 52.59, 38.93, 25.94, 23.23. HRMS C<sub>5</sub>H<sub>14</sub>N<sub>2</sub>O calculated [M+H]<sup>+</sup> 119.117876. found: 119.1185.



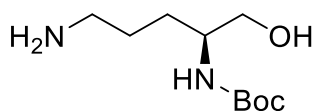
Compound **2a** – (S)-2,5-diaminohexan-1-ol



**2a**

Synthesised from Z-Lys(Boc)-OH using step (i) and (iii) consecutively. Brown oil (97 mg, 59 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>) δ = 3.88 – 3.62 (m, 2H), 3.30 (qd, J=6.7, 3.5, 1H), 3.03 (t, J=7.6, 2H), 1.84 – 1.68 (m, 4H), 1.63 – 1.53 (m, 2H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>) δ = 60.53, 52.97, 39.00, 28.44, 26.84, 22.05. HRMS C<sub>6</sub>H<sub>16</sub>N<sub>2</sub>O calculated [M+H]<sup>+</sup> 133.133576. found: 133.1340.

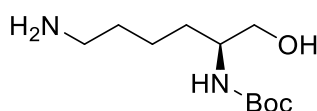
Compound **1b** – tert-butyl (S)-(5-amino-1-hydroxypentan-2-yl)carbamate



**1b**

Synthesised from Boc-Orn(Z)-OH following steps (i) and (iii) consecutively. Brown oil (42 mg, 47 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>) δ = 3.54 – 3.38 (m, 3H), 3.30 (p, J=1.6, 2H), 2.69 – 2.60 (m, 2H), 1.63 – 1.27 (m, 13H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>) δ = 156.93, 78.53, 63.97, 52.15, 40.91, 28.61, 28.26, 27.37. HRMS C<sub>10</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> calculated [M+H]<sup>+</sup> 219.170276. found: 219.1706.

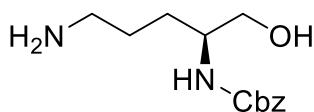
Compound **2b** – tert-butyl (S)-(6-amino-1-hydroxyhexan-2-yl)carbamate



**2b**

Synthesised from Boc-Lys(Z)-OH following steps (i) and (iii) consecutively. Brown oil (42.3 mg, 44 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>) δ = 3.51 – 3.41 (m, 4H), 3.30 (p, J=1.6, 1H), 2.63 (t, J=7.1, 2H), 1.60 – 1.31 (m, 15H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>) δ = 156.96, 78.49, 63.99, 52.24, 40.89, 31.98, 30.73, 27.38, 22.95. HRMS C<sub>11</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub> calculated [M+H]<sup>+</sup> 233.185976. found: 233.1861.

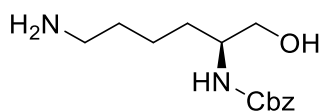
Compound **1c** – benzyl (S)-(5-amino-1-hydroxypentan-2-yl)carbamate



**1c**

Synthesised from Z-Orn(Boc)-OH following steps (i) and (ii) consecutively. White solid powder (80.3 mg, 78 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>) δ = 7.47 – 7.17 (m, 5H), 5.08 (s, 2H), 3.69 – 3.56 (m, 1H), 3.51 (dd, J=5.6, 3.3, 2H), 3.31 (s, 1H), 2.94 (dt, J=8.5, 6.2, 2H), 1.85 – 1.60 (m, 3H), 1.52 – 1.42 (m, 1H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>) δ = 157.45, 136.91, 128.08, 127.61, 127.42, 66.08, 63.73, 52.33, 39.15, 27.84, 23.83. HRMS C<sub>13</sub>H<sub>20</sub>N<sub>2</sub>O<sub>3</sub> calculated [M+H]<sup>+</sup> 253.154676. found: 253.1548.

Compound **2c** – benzyl (S)-(6-amino-1-hydroxyhexan-2-yl)carbamate



**2c**

Synthesised from Z-Lys(Boc)-OH following steps (i) and (ii) consecutively. White solid powder (96 mg, 61 %) <sup>1</sup>H NMR (400 MHz, Methanol-d<sub>4</sub>) δ = 7.40 – 7.17 (m, 5H), 5.06 (s, 2H), 3.60 (dd, J=8.9, 4.7, 1H), 3.54 – 3.45 (m, 2H), 3.30 (t, J=1.7, 1H), 2.99 – 2.76 (m, 2H), 1.72 – 1.29 (m, 7H). <sup>13</sup>C NMR (101 MHz, Methanol-d<sub>4</sub>) δ = 157.45, 137.00, 128.06, 127.58, 127.39, 66.00, 63.82, 52.54, 39.24, 30.35, 26.92, 22.50. HRMS C<sub>10</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub> calculated [M+H]<sup>+</sup> 267.170276. found: 267.1699.

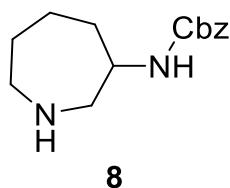
## Synthesis of product standard: 3-Cbz-aminoazepane (8)

### Cbz-protection of 1-Boc-N-3-aminoazepane

To a solution of 1-Boc-N-3-aminoazepane (857 mg, 4 mmol) in DCM (10 mL), N,N-Diisopropylethylamine (DIEA) (1.04 mL, 6 mmol) and benzyl chloroformate (0.72 mL, 5 mmol) were added at 0 °C and stirred. The reaction mixture was stirred at room temperature until TLC monitoring showed that the reaction was complete. The mixture was diluted with DCM and washed with water and brine. The organic layer was dried over anhydrous MgSO<sub>4</sub> and concentrated on rotary evaporator. The crude product was purified by flash column chromatography (hexane/ethyl acetate = 6:1) to yield the product, 1-Boc-3-Cbz-N-aminoazepane as clear oil.

1-Boc-3-Cbz-N-3-aminoazepane (0.5011 g, 1.438 mmol) was suspended DCM (5 mL) at 0 °C and TFA (2.5 mL) was added dropwise. The reaction mixture was stirred at 0 °C and progress was monitored by TLC (6:1, Hexane: EtOAc). After 2 hours, TLC suggested the reaction was complete. The reaction mixture was concentrated and the crude residue was dissolved in water (50 mL). The aqueous layer was washed with EtOAc (2 x 30 mL), then basified to pH 9 at 0 °C with NaHCO<sub>3</sub>. Thereafter the aqueous layer was extracted with CHCl<sub>3</sub> (3 x 50 mL). This second organic layer was washed with water (50 mL), brine (10 mL) and dried over MgSO<sub>4</sub>. The product was concentrated on rotary evaporator.

Compound 8 – benzyl azepan-3-ylcarbamate



Yellow solid (167.3 mg, 47 %) <sup>1</sup>H NMR (400 MHz, Chloroform-d)  $\delta$  = 7.34 – 7.18 (m, 5H), 5.01 (s, 2H), 3.86 – 3.60 (m, 1H), 2.92 – 2.41 (m, 5H), 1.80 – 1.10 (m, 7H). <sup>13</sup>C NMR (101 MHz, Chloroform-d)  $\delta$  = 155.62, 136.74, 128.50, 128.11, 128.03, 66.45, 52.99, 51.02, 49.13, 34.31, 29.95, 21.86. HRMS C<sub>14</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub> calculated [M+H]<sup>+</sup> 249.159776. found: 249.1605.

## Analytical Methods

### Reverse phase HPLC

**Table S4:** 85:10 isocratic method for HPLC analysis of PTSC derived L-3-aminopiperidine. Each solvent contained 0.1 % TFA

Time [min]	Water (H <sub>2</sub> O) [%]	Acetonitrile (AcN) [%]
0	85	15
10	85	15

## Normal phase (chiral) HPLC

**Table S5:** 70:30 isocratic method for chiral HPLC analysis of L-Cbz-3-aminopiperidine (**7c**) and L-Cbz-3-aminoazepane (**8c**). Each solvent contained 0.1% DEA

Time [min]	Hexane (C <sub>6</sub> H <sub>14</sub> ) [%]	Iso-propanol (C <sub>3</sub> H <sub>7</sub> OH) [%]
0	70	30
20	70	30

## GC-FID

**Table S6:** 30 °C min<sup>-1</sup> gradient method for GC-FID analysis of L-Boc-3-aminopiperidine, L-Boc-3-aminoazepane, L-Cbz-3-aminopiperidine (**7**) and L-Cbz-3-aminoazepane (**8**). Injection volume: 2 µL, Injection split 1:10, detector temperature 325 °C.

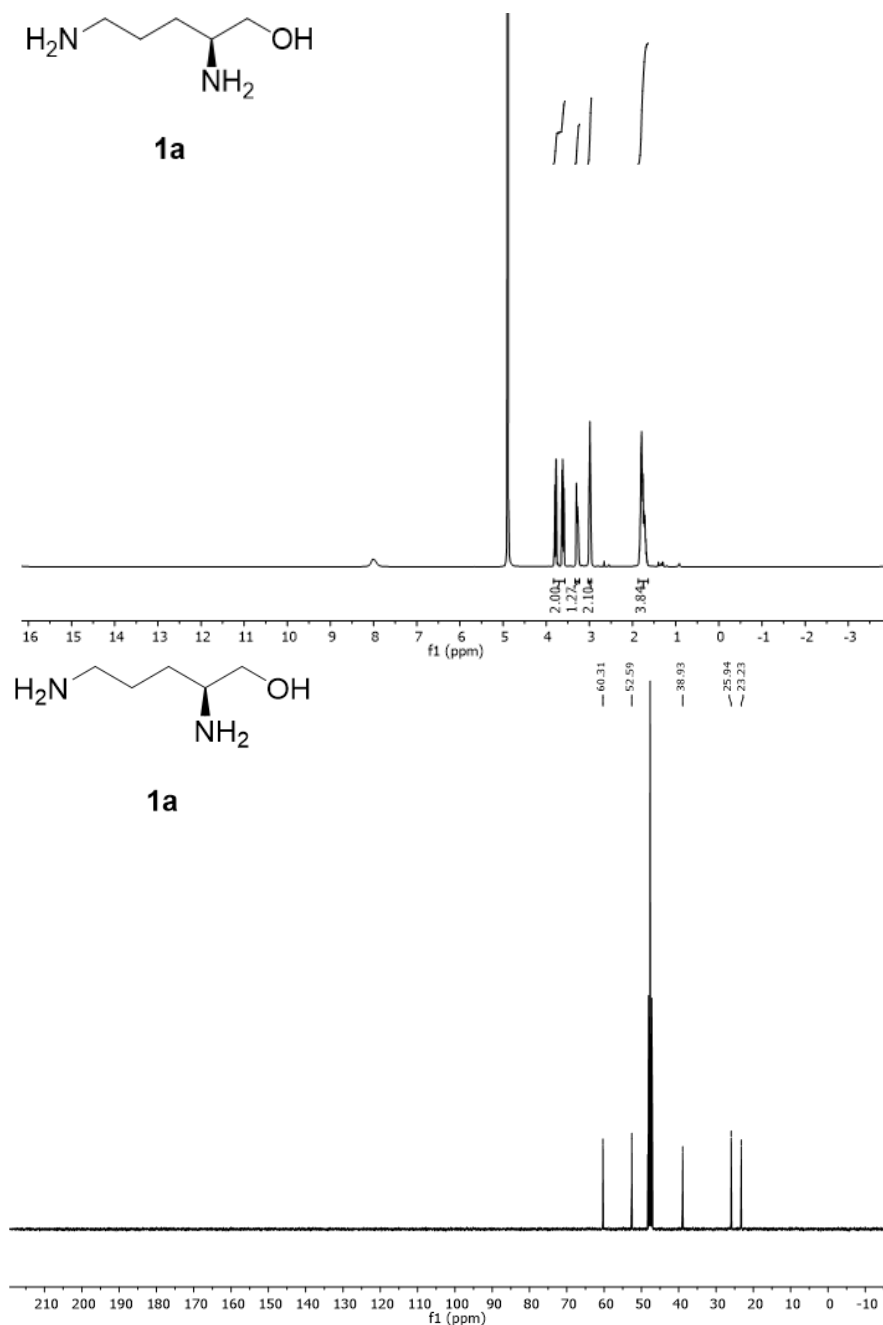
Time [min]	Temperature [°C]	Temperature gradient [°C min <sup>-1</sup> ]
0	100	0
2	100	30
8.66	300	0

**Table S7:** 30 °C min<sup>-1</sup> gradient method for GC-MS analysis of biotransformation samples to identify benzyl alcohol. Injection volume: 2 µL, Injection split 1:10, detector temperature 325 °C.

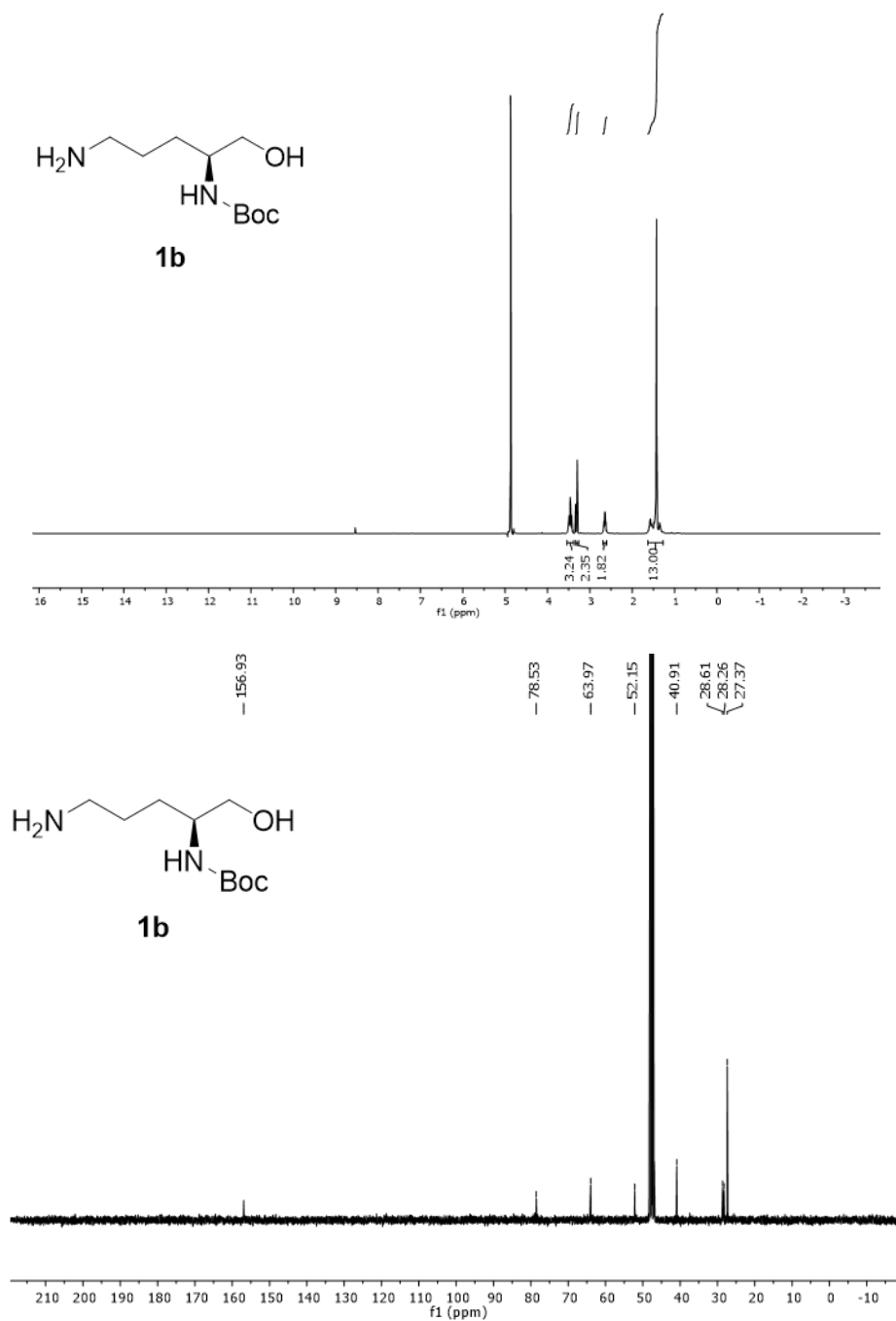
Time [min]	Temperature [°C]	Temperature gradient [°C min <sup>-1</sup> ]
0	100	0
3	100	30
9.66	300	0

# Characterization Data

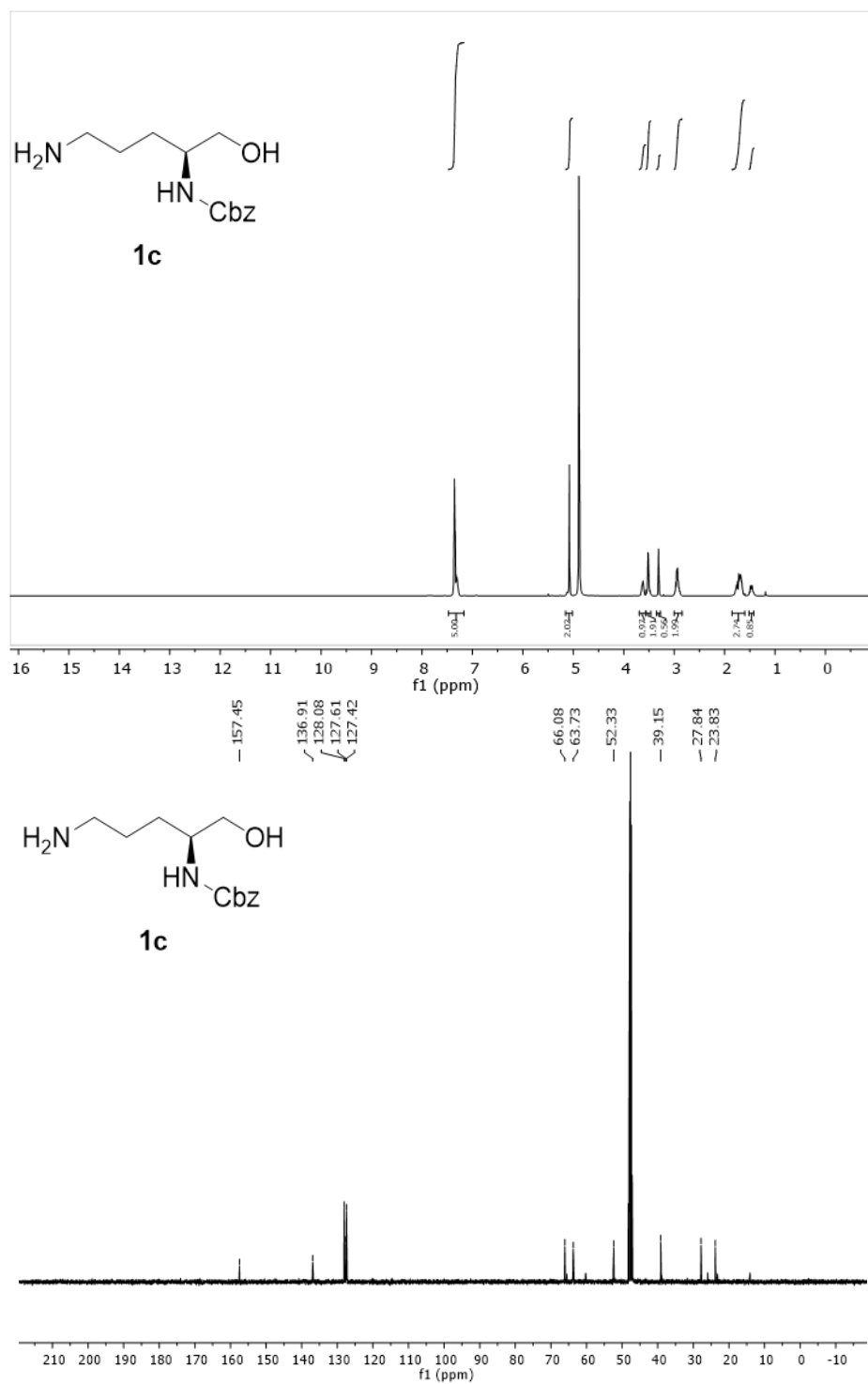
## Chemical synthesis – NMR



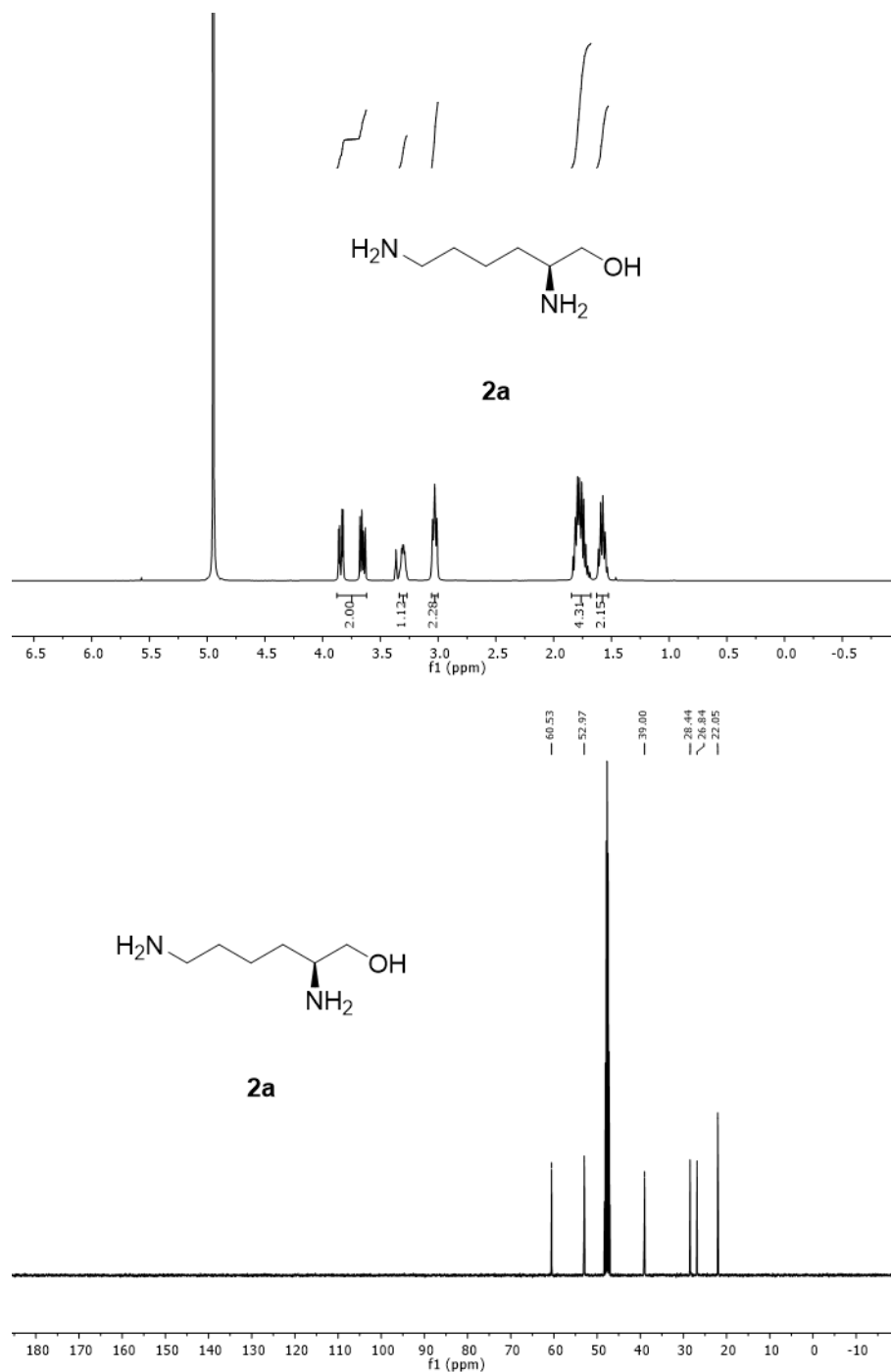
**Figure S10:** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of chemically synthesised **1a** which was used as a substrate for biotransformations.



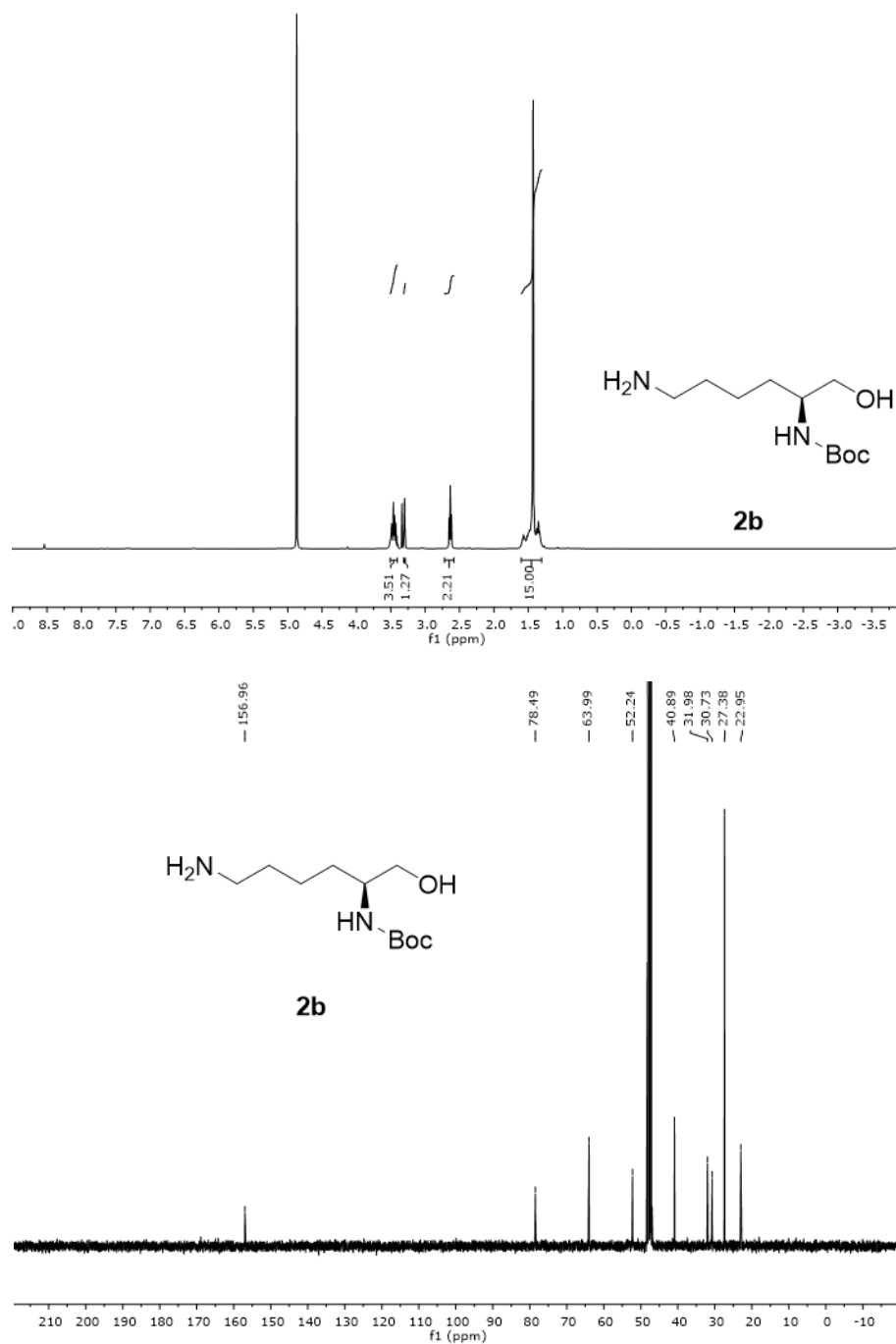
**Figure S11:** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of chemically synthesised **1b** which was used as a substrate for biotransformations.



**Figure S12:** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of chemically synthesised **1c** which was used as a substrate for biotransformations.

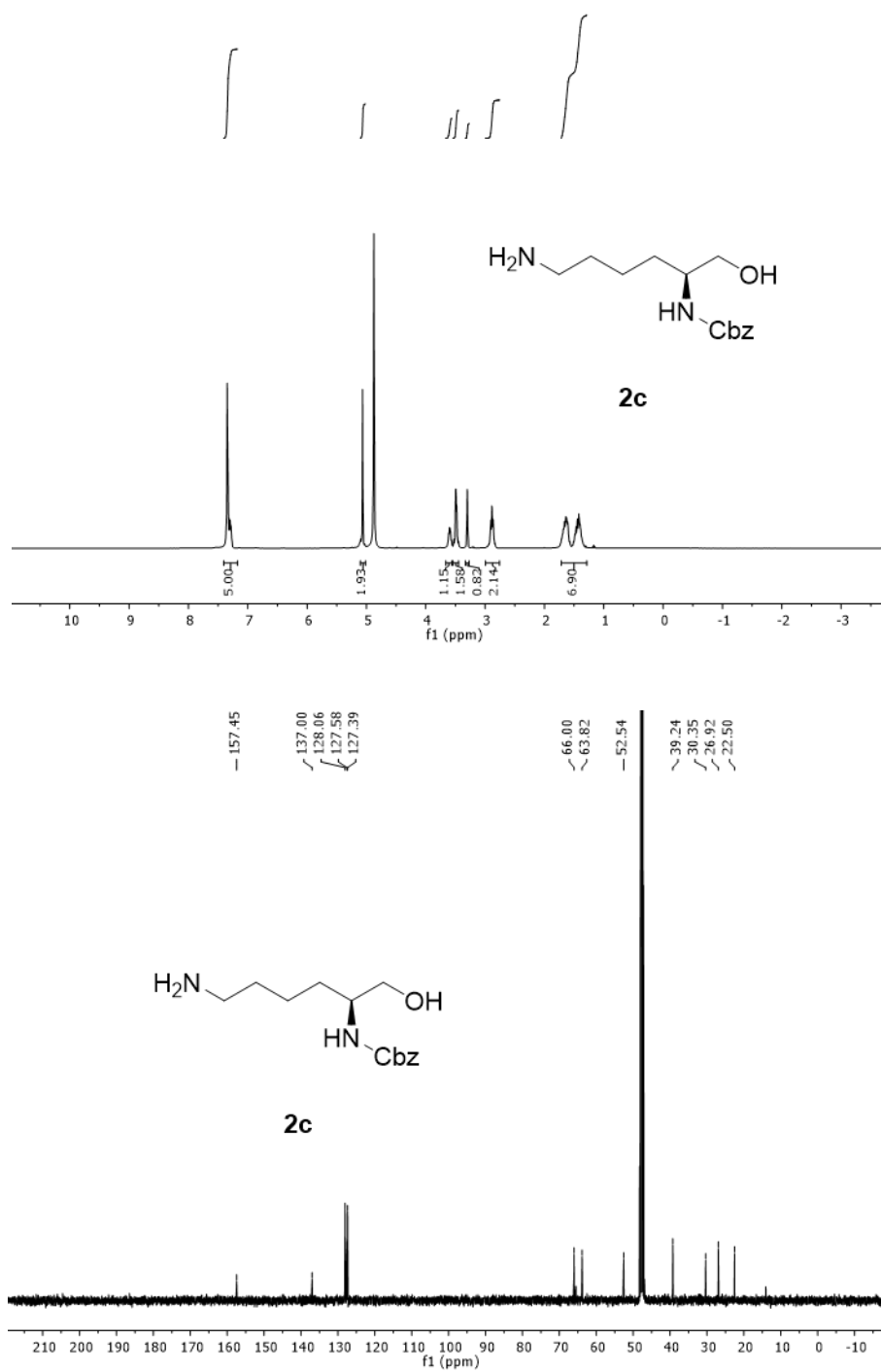


**Figure S13:**  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR of chemically synthesised **2a** which was used as a substrate for biotransformations.

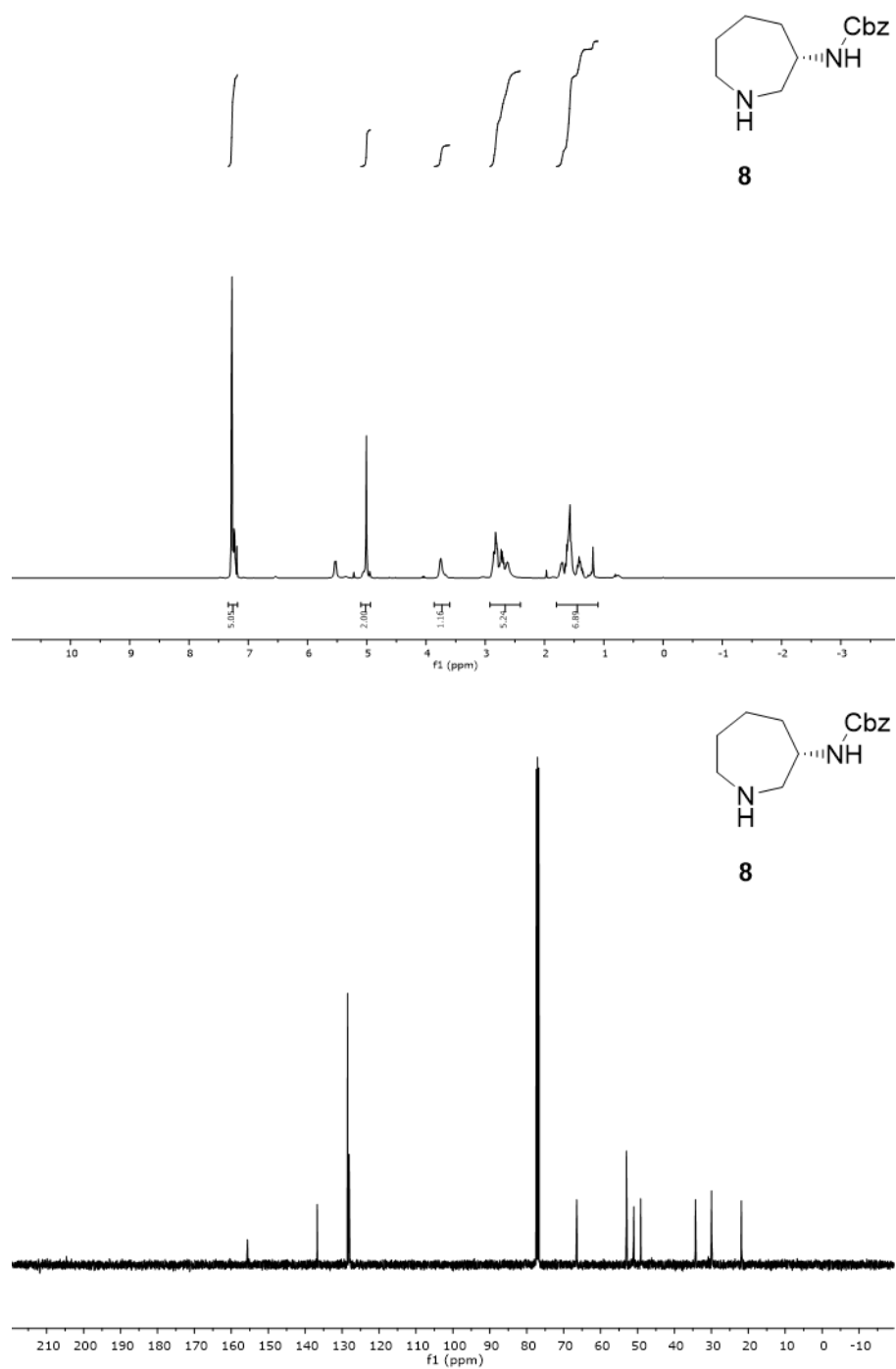


**Figure S14:**  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR of chemically synthesised **2b** which was used as a substrate for biotransformations.



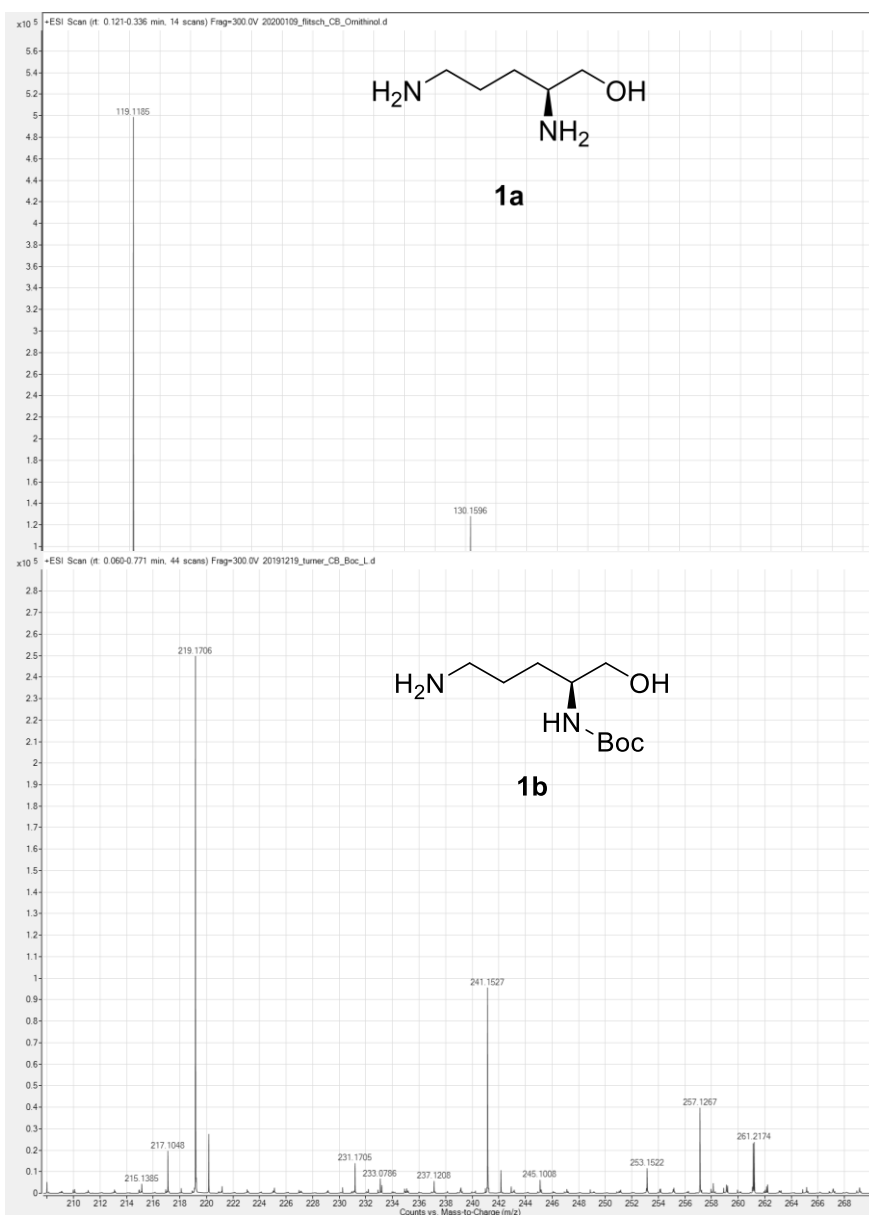


**Figure S15:** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR of chemically synthesised **2c** which was used as a substrate for biotransformations.

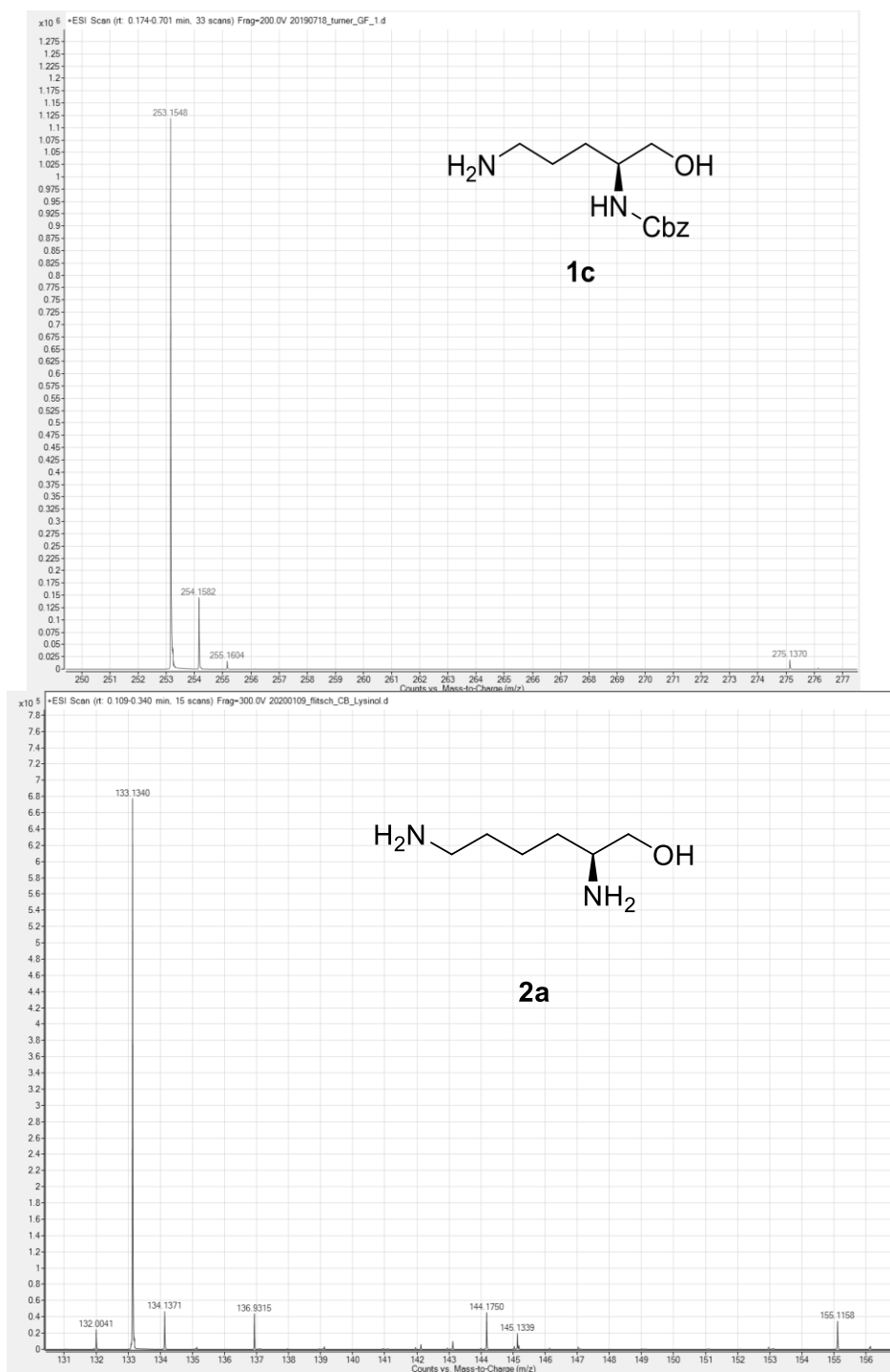


**Figure S16:**  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  of chemically synthesised **8c** which was used as a chemical standard.

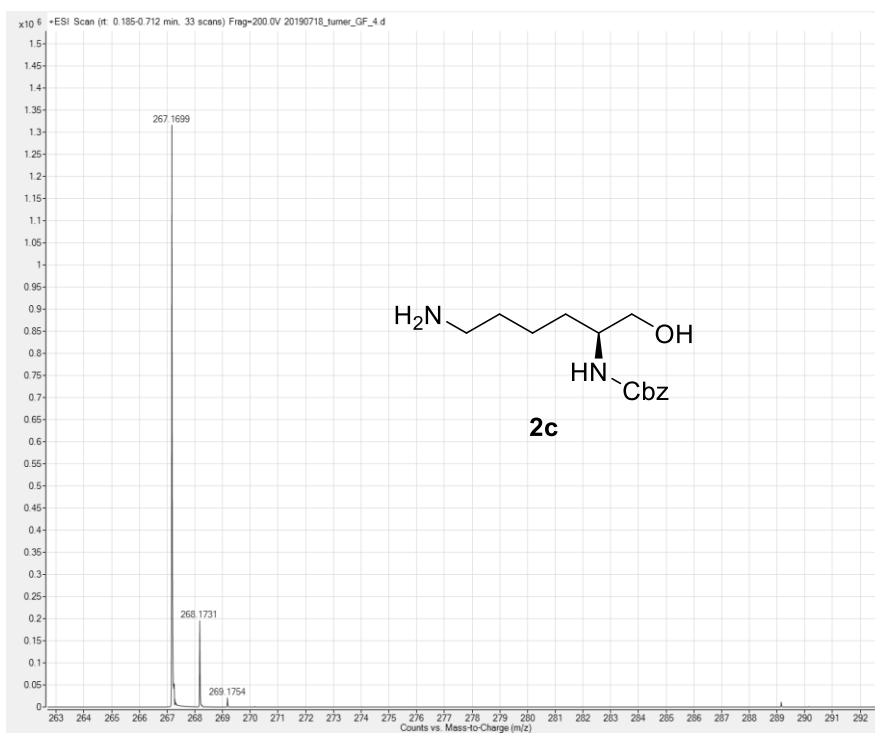
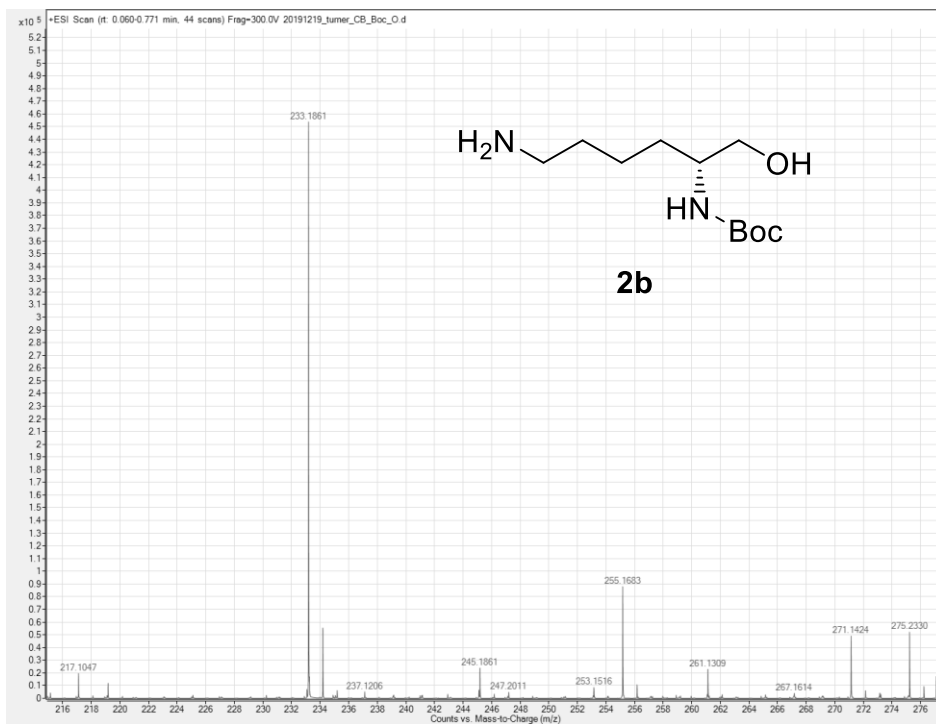
## Chemical synthesis - HRMS



**Figure S17:** HRMS of chemically synthesised **1a** and **1b** which were used as substrates for biotransformations.

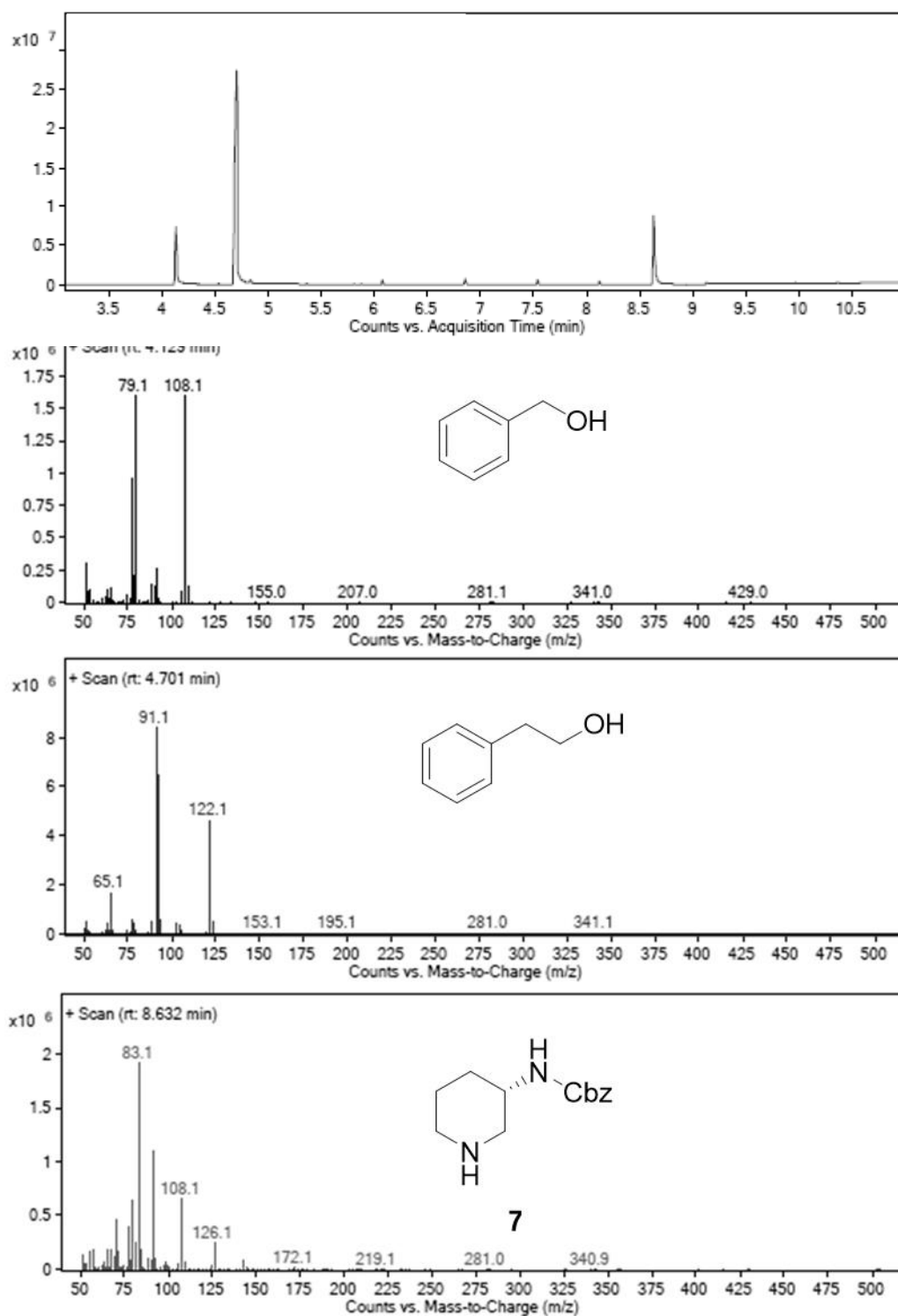


**Figure S18:** HRMS of chemically synthesised **1c** and **2a** which were used as substrates for biotransformations.



**Figure S19:** HRMS of chemically synthesised **2b** and **2c** which were used as substrates for biotransformations.

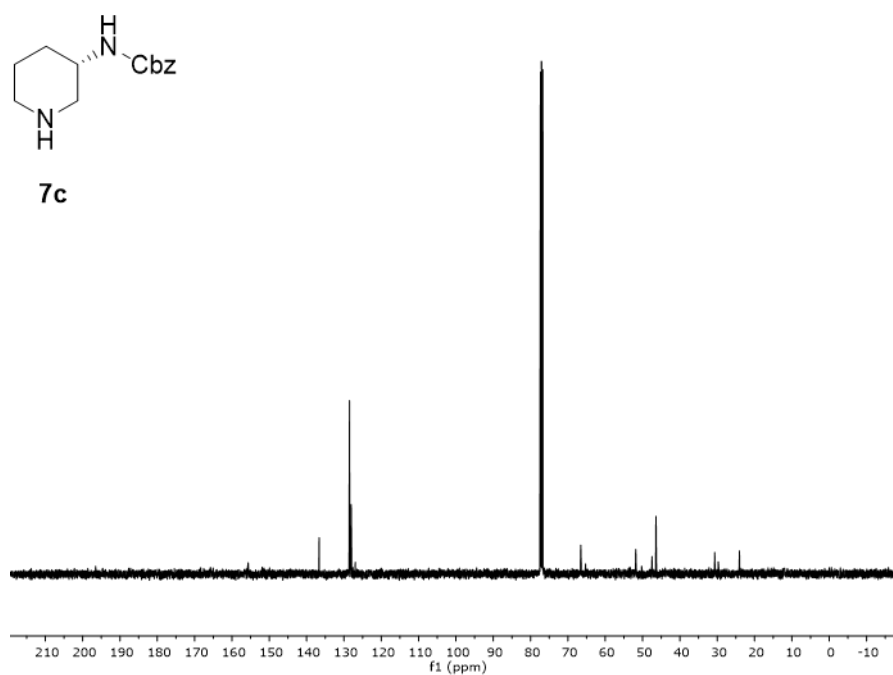
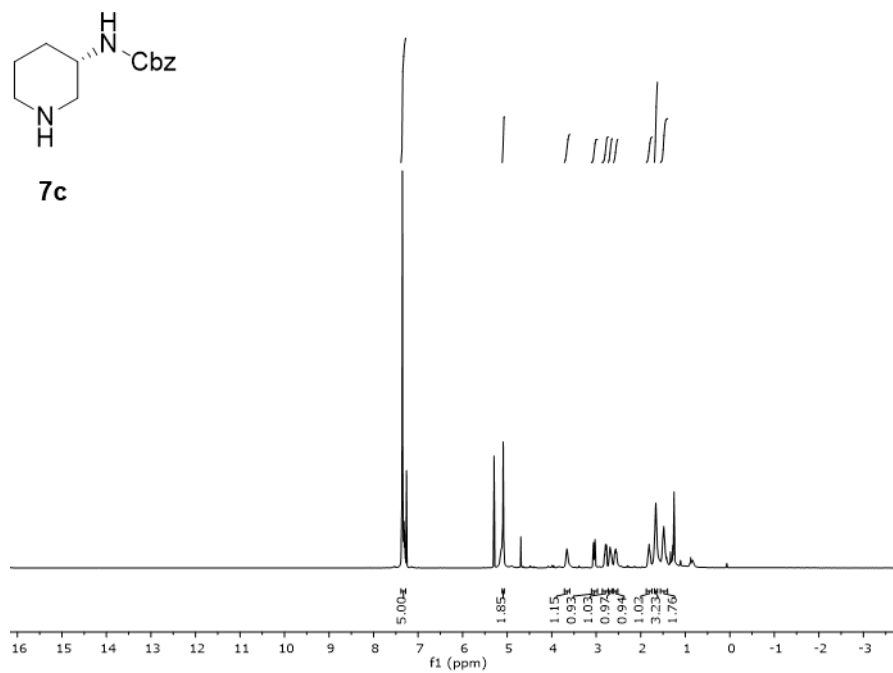
## Identification of benzyl alcohol formation in biotransformation analysis



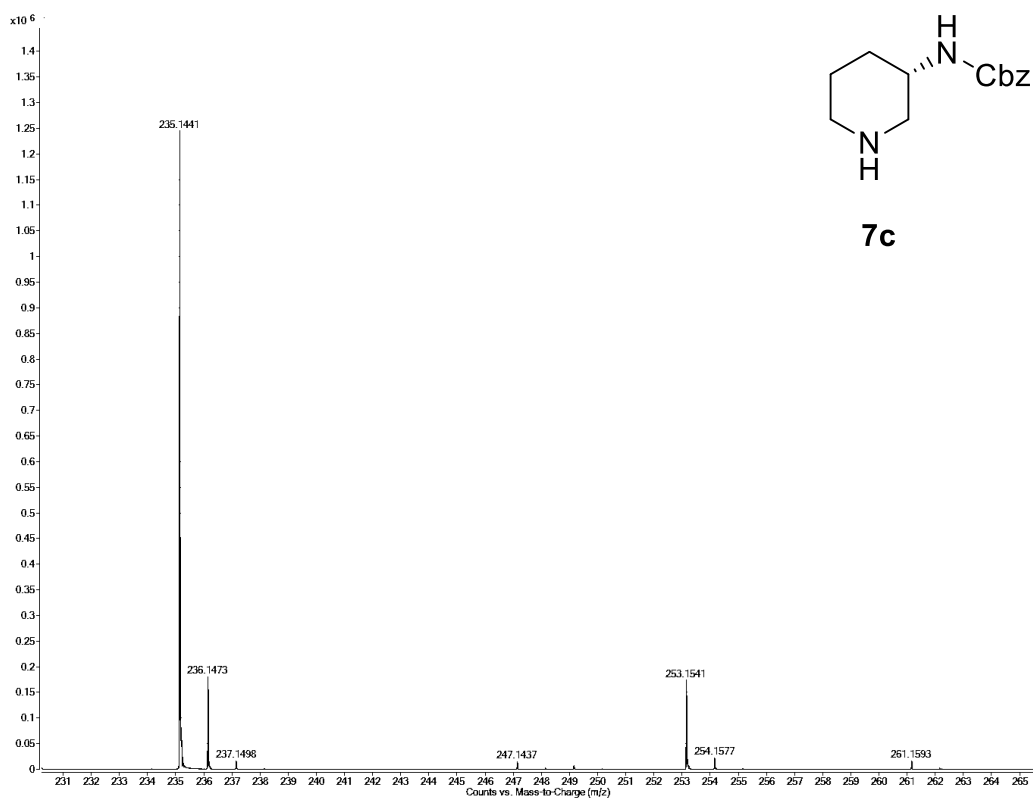
**Figure S20:** GC-MS from analytical biotransformation of **1c** to **7** identifying benzyl alcohol formation.

## Biotransformation Scale-up

### System 1



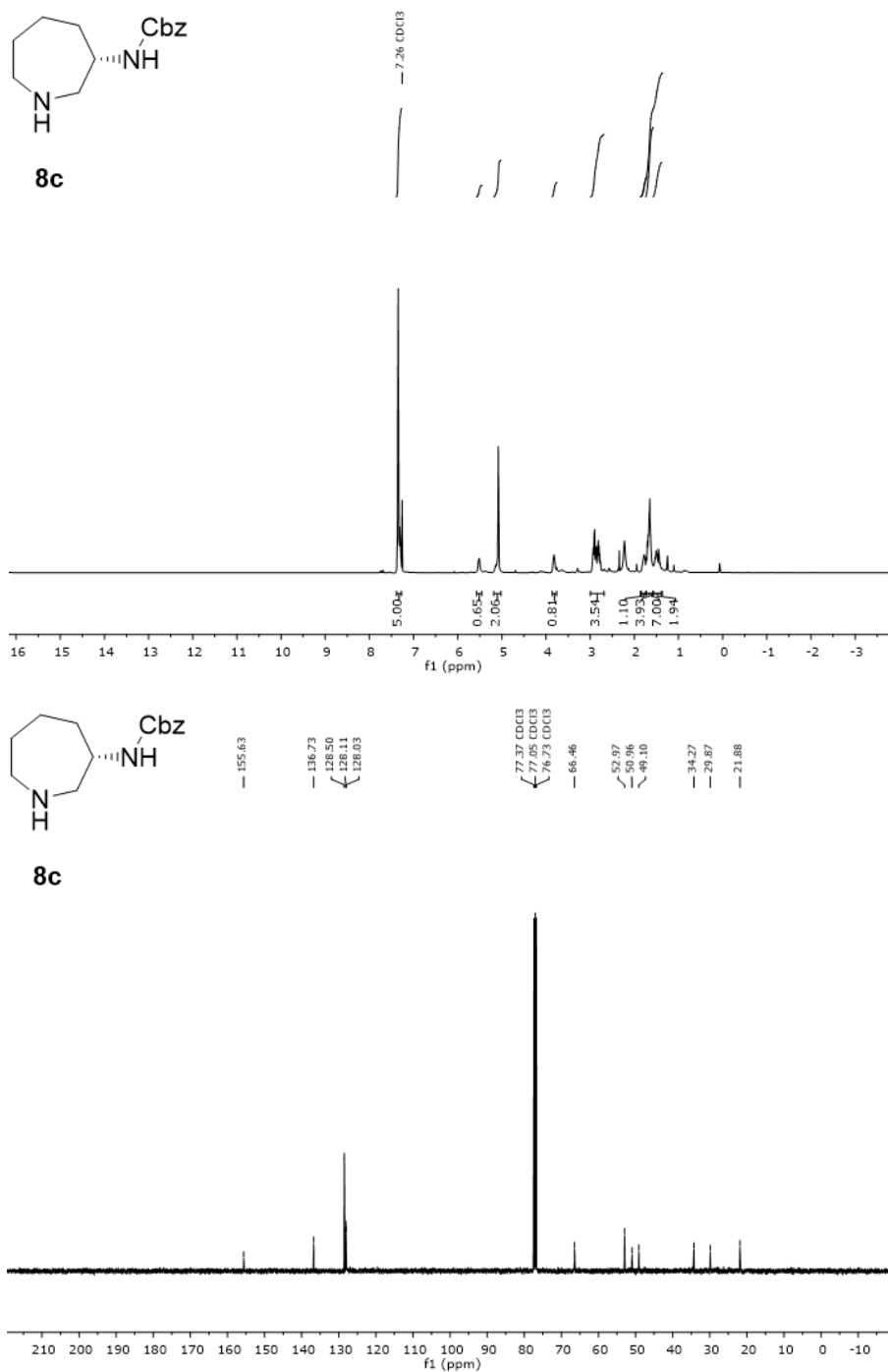
**Figure S21:**  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR of **7** yielded from scaled-up biotransformation of substrate **1c** with GOase M<sub>3-5</sub> and AdRedAm.



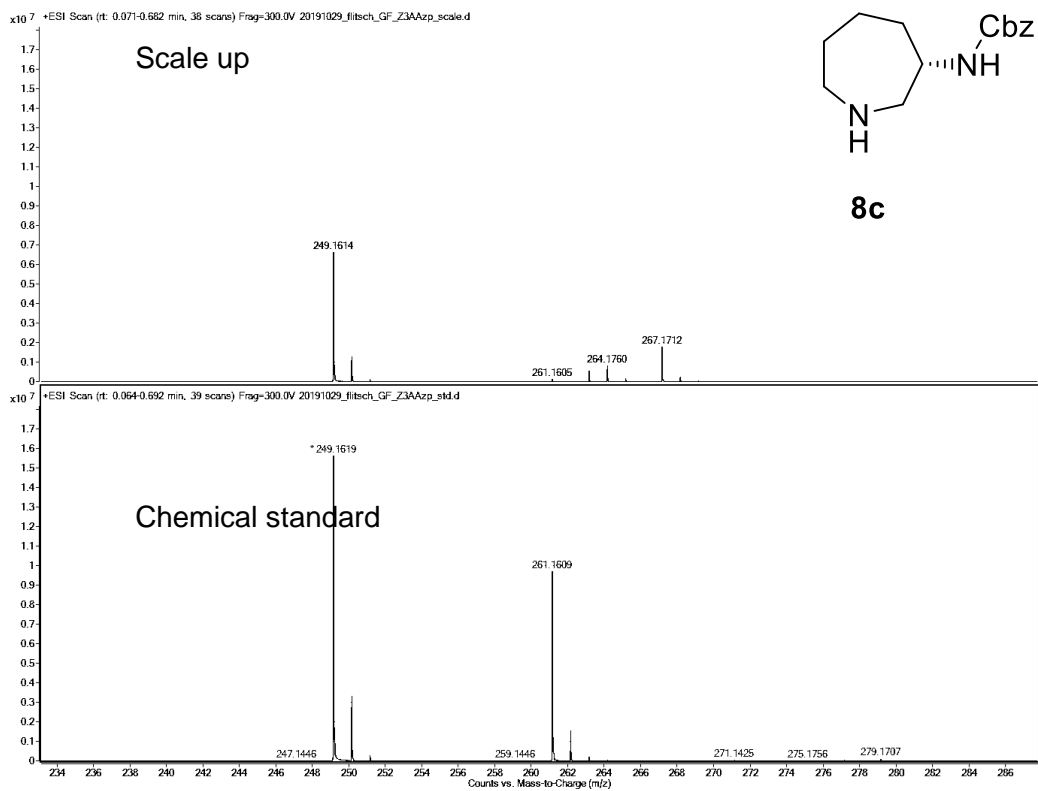
**Figure S22:** HRMS of **7** yielded from scaled-up biotransformation of substrate **1c** with GOase *M*<sub>3-5</sub> and *AdRedAm*.



System 2



**Figure S23:**  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR of **8** yielded from scaled-up biotransformation of substrate **2c** with GOase M<sub>3-5</sub> and IR-49.



**Figure S24:** HRMS of **8** from (top) scaled-up biotransformation of substrate **2c** with GOase M<sub>3-5</sub> and IR-49 and (bottom) chemical synthesis.

## Reverse phase HPLC

Authentic standard (PTSC derived)

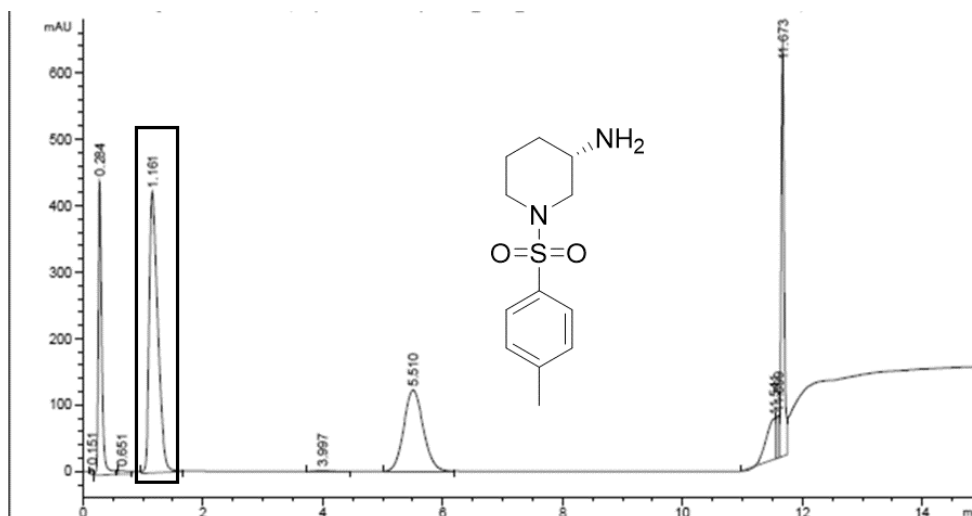


Figure S25: HPLC trace for PTSC derived L-3-aminopiperidine product standard.

Initial biotransformation trial (PTSC derived)

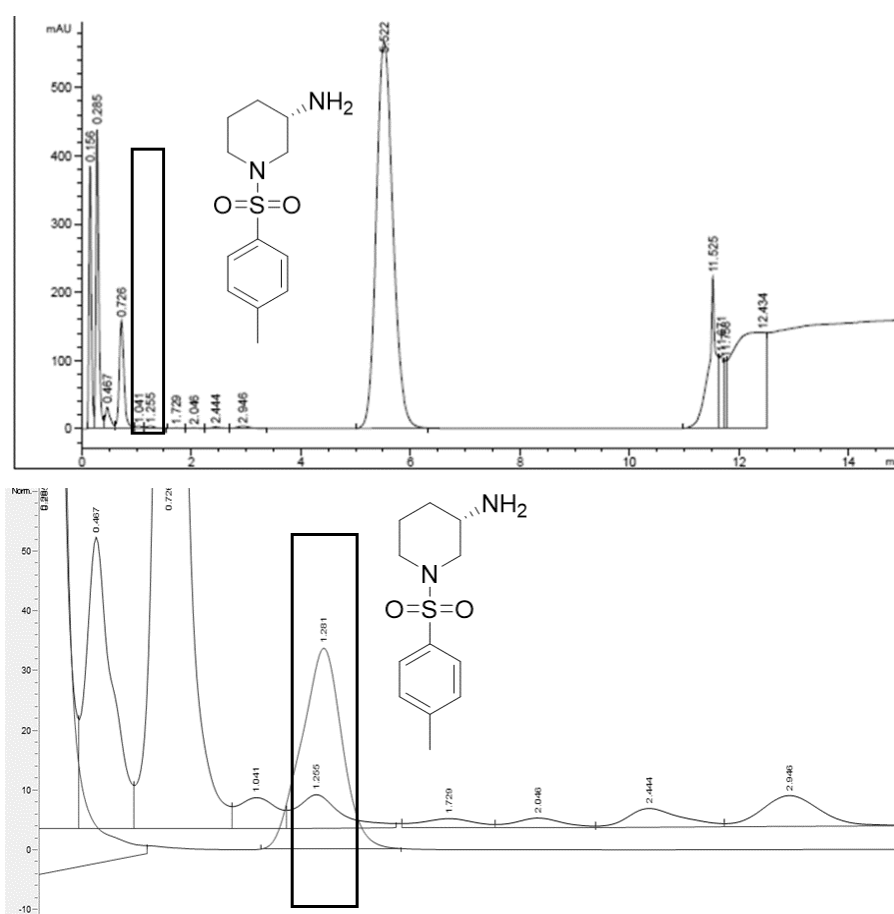
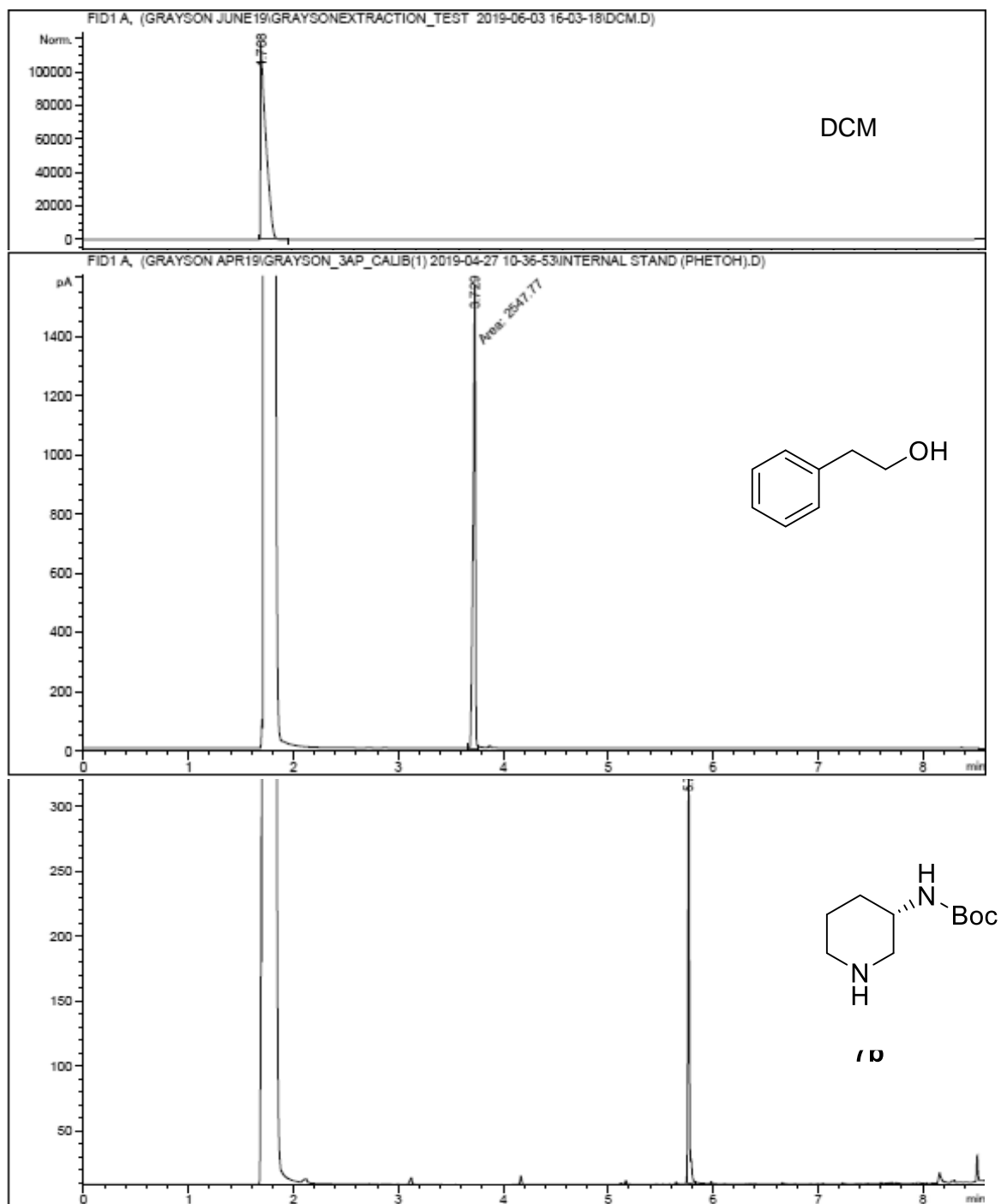


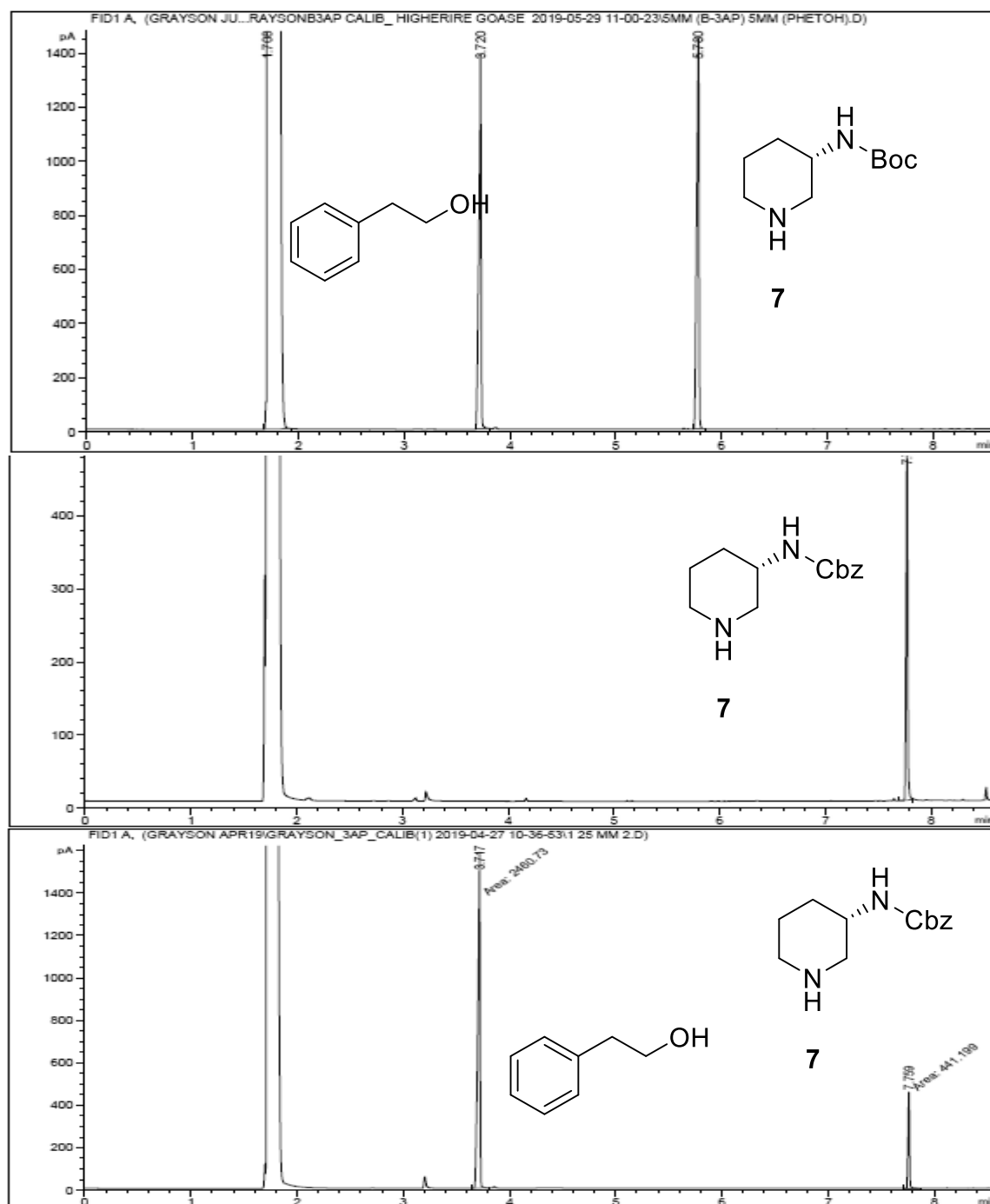
Figure S26: HPLC for PTSC derived L-3-aminopiperidine from initial biotransformation trials.

## GC-FID Traces

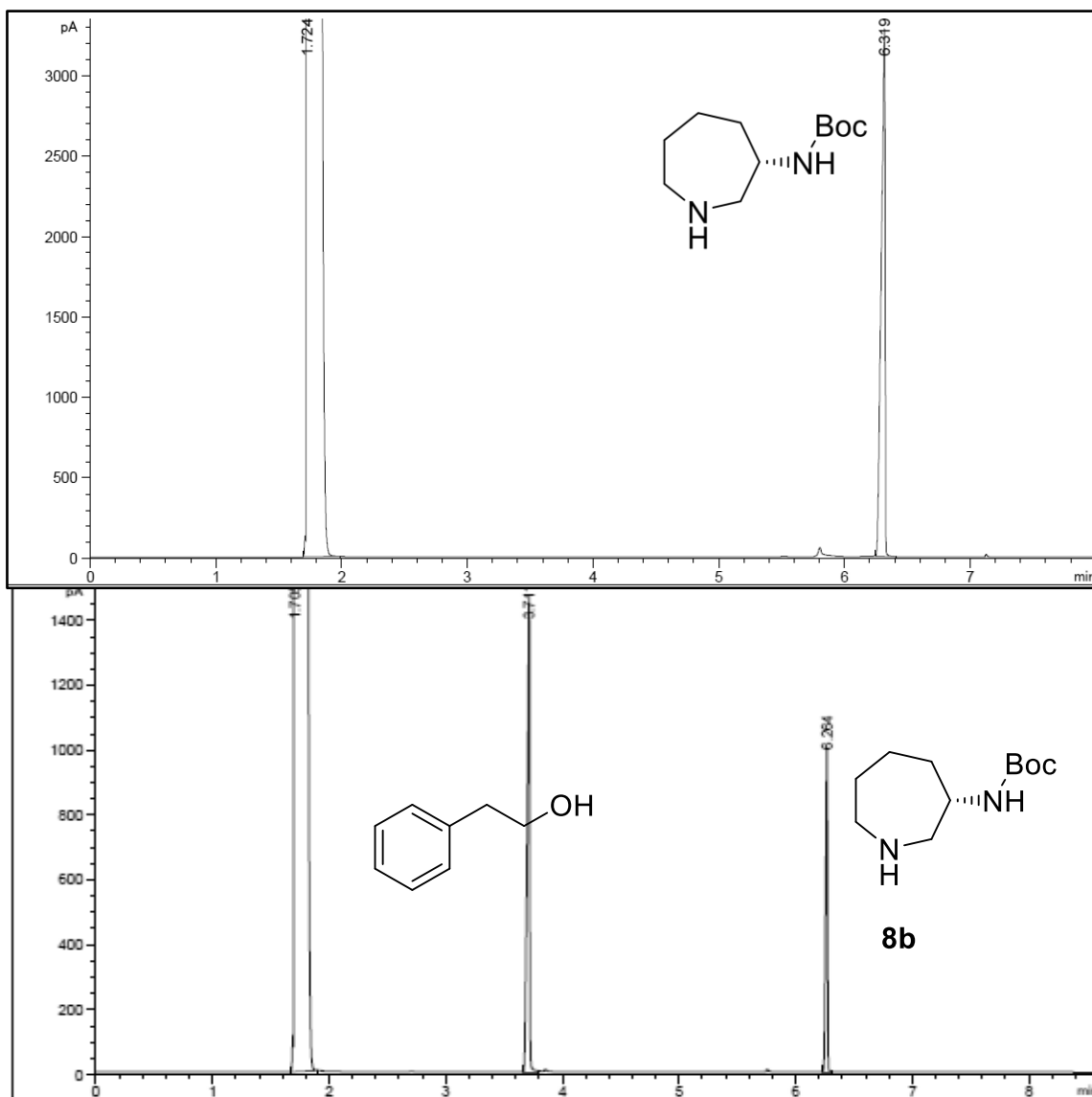
### Authentic Standards



**Figure S27:** GC-FID traces of (top) biotransformation extraction solvent- DCM, (middle) phenylethanol (PhEtOH) used as an internal standard to quantify product formed and (bottom) product standard of L-Boc-3-aminopiperidine.

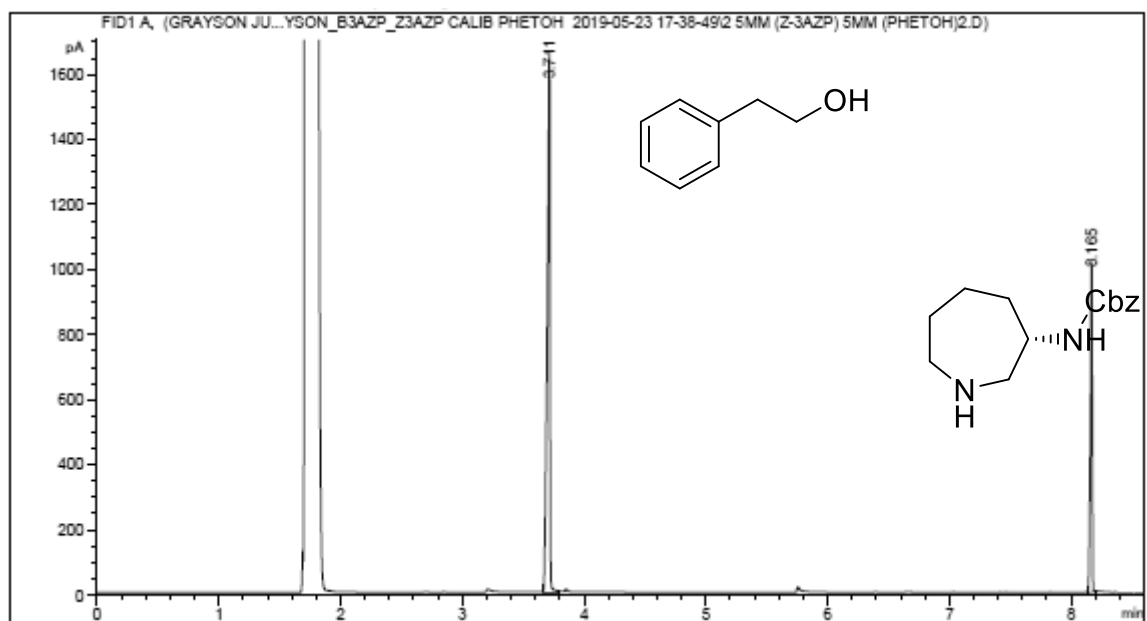


**Figure S28:** GC-FID traces of (top) L-Boc-3-aminopiperidine standard with internal standard PhEtOH (middle) product standard of L-Cbz-3-aminopiperidine (**7**) and (bottom) product standard of **7** with internal standard PhEtOH.



**Figure S29:** GC-FID trace of (top) product standard **8b** and (bottom) **8b** without internal standard PhEtOH.

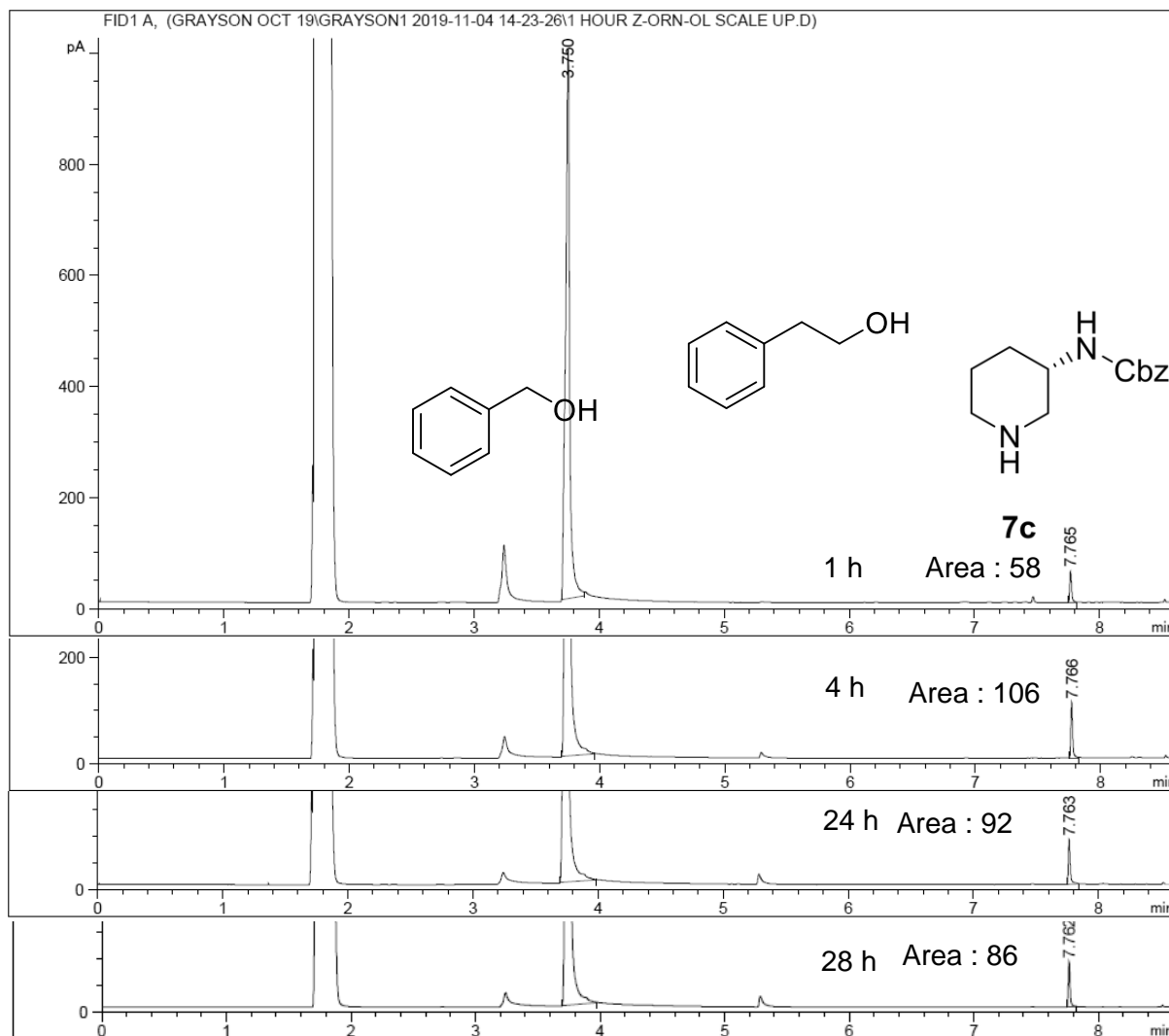
## Chemically synthesised standard



**Figure S30:** GC-FID trace of chemically synthesised product standard **8c** with internal standard PhEtOH.

## Biotransformation scale up

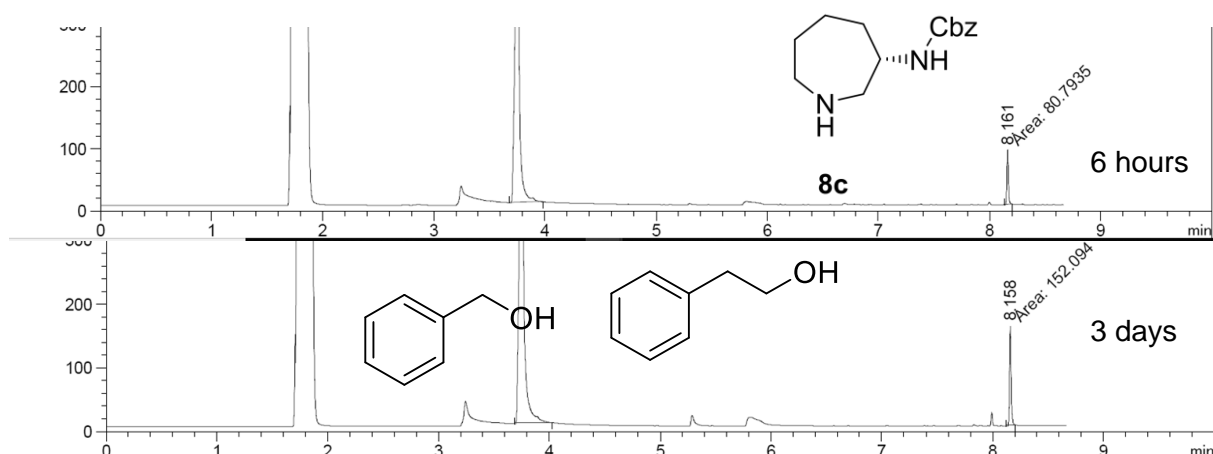
### System 1 – scale up



**Figure S31:** GC-FID traces of scaled-up biotransformation for the conversion of substrate **1c** to product **7** using GOase M<sub>3-5</sub> and AdRedAm.



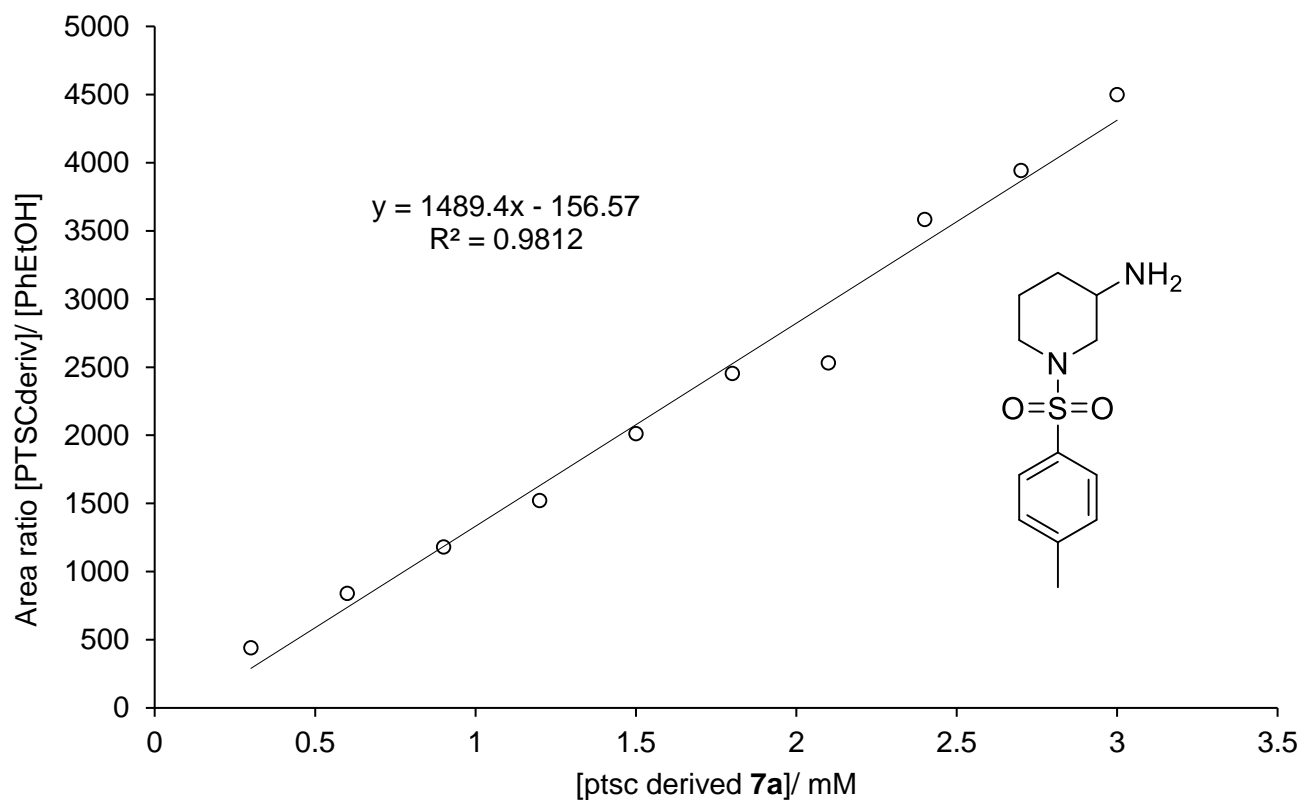
## System 2 – scale up



**Figure S32:** GC-FID traces of scaled-up biotransformation for the conversion of substrate **2c** to product **8** using GOase M<sub>3-5</sub> and IR-49.

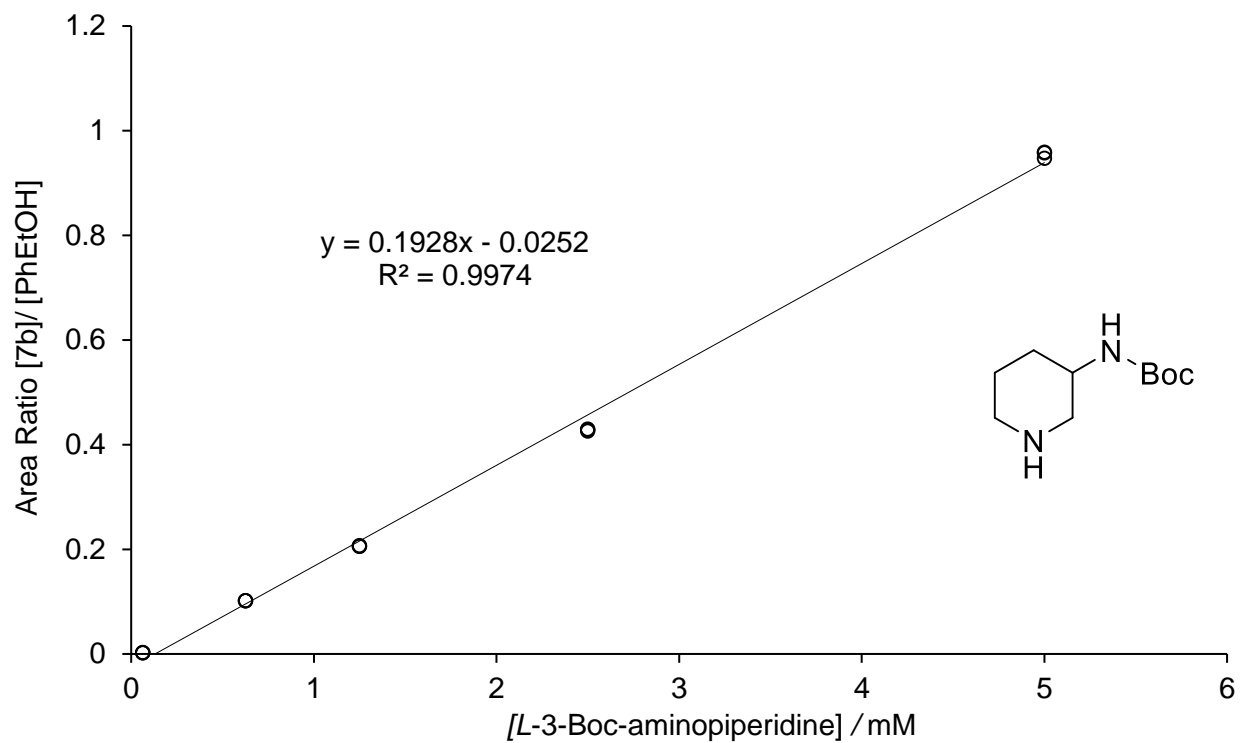
## Calibration curves

### Reverse phase HPLC

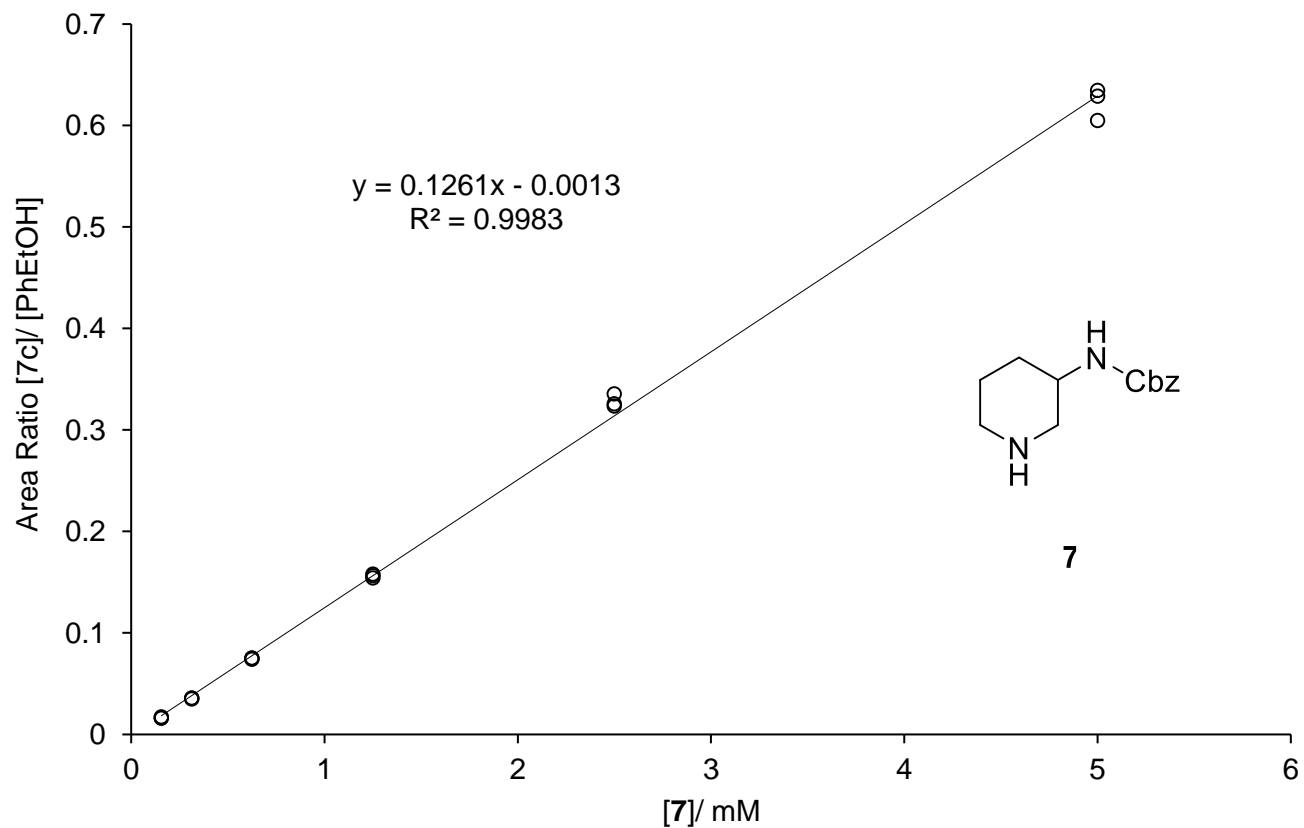


**Figure S33:** Calibration curve for 3-aminopiperidine after derivatisation with para-toulenesulfonyl chloride (PTSC).

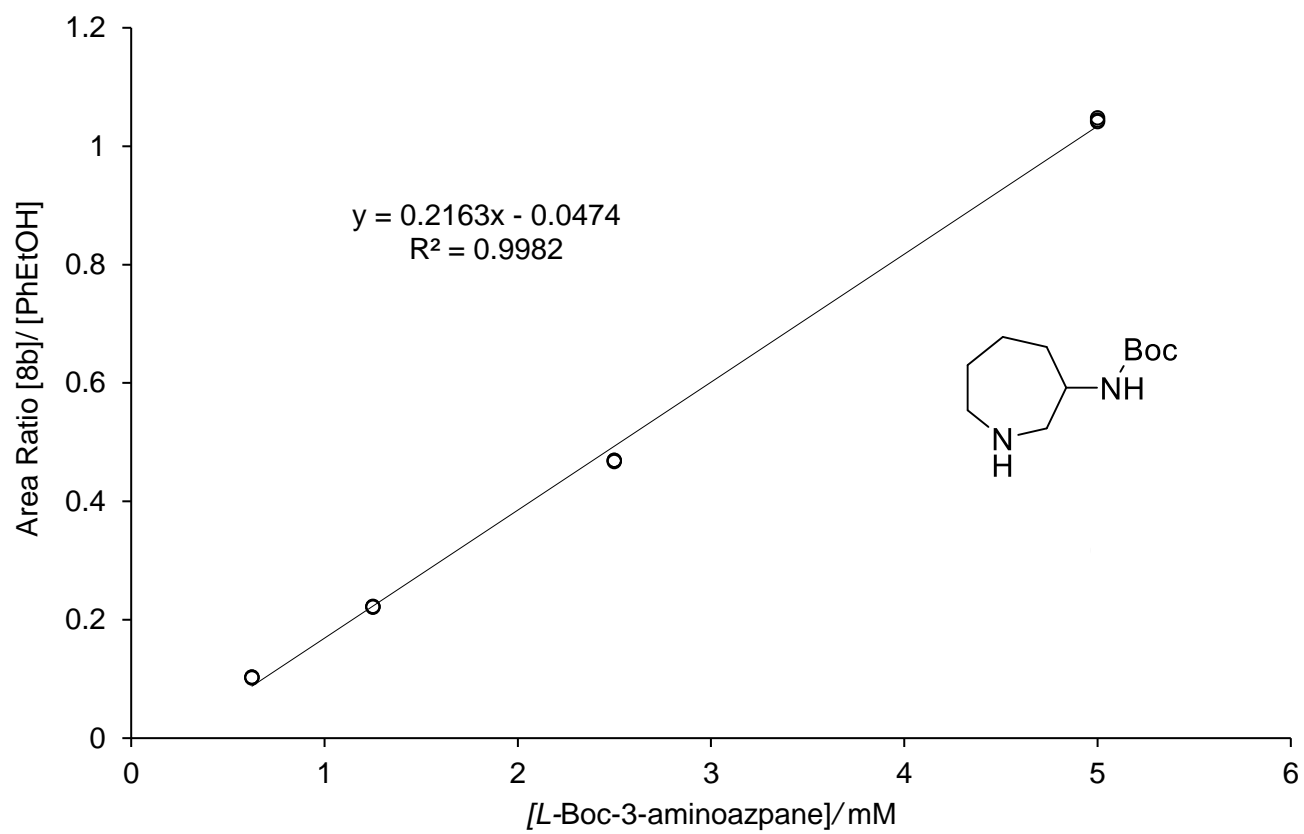
### GC-FID



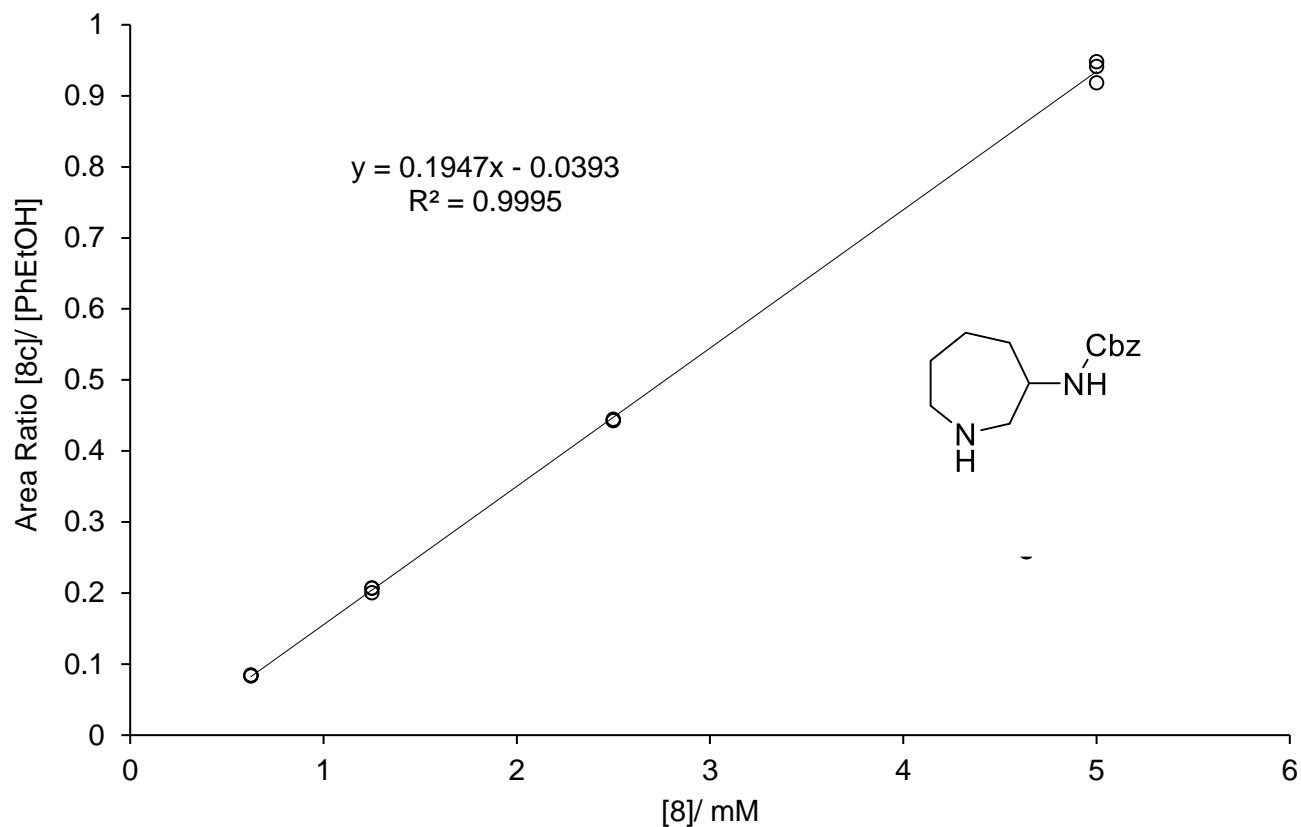
**Figure S34:** Calibration curve for Boc-3-aminopiperidine.



**Figure S35:** Calibration curve for Cbz-3-aminopiperidine (**7**).



**Figure S36:** Calibration curve for Boc-3-aminoazepane.



**Figure S37:** Calibration curve of Cbz-3-aminoazepane (**8**).

## References

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