Supporting Information
A biocatalytic cascade for the amination of unfunctionalised cycloalkanes

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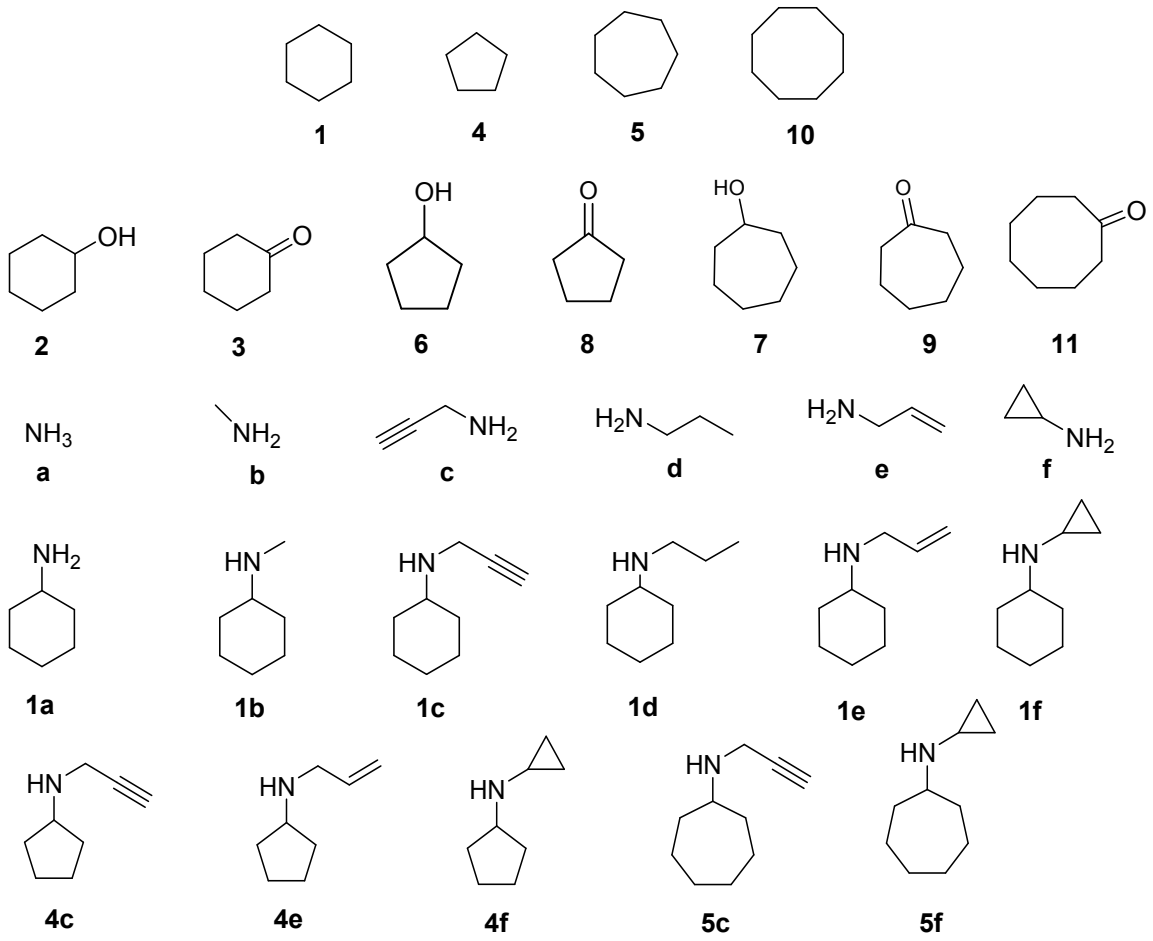


List of substrates and products mentioned in the text.

Experimental

Materials

Solvents, commercially available chemicals and carbon monoxide for CO difference spectroscopy were obtained from Sigma-Aldrich (Poole, Dorset, UK). Gases for GC-FID analysis were purchased from BOC gases (Guildford, UK). Chemically competent cells and enzymes for molecular biology were purchased from New England Biolabs (Hitchin, UK). Terrific Broth Base autoinduction medium including trace elements (TB-AIM) was purchased from Formedium (Hunstanton, UK).

Molecular biology methods

Custom primer synthesis and plasmid DNA sequencing were performed by Eurofins Genomics. Primers employed in this work are given below (mismatching bases are given in red).

Primer	**5'->3' sequence**
R47L Y51F for, Tm=66°C	AGGCGCCTGGTCTGGTAACGCGCTTCTTATCAAGTCAGCGTC
R47L Y51F rev, Tm=63°C	CGAATTTAAAGATTTCTCCTAATTCATCCGCAATTTTCATCAAAGC
R966D for, Tm=60°C	CCGCTTTTTCTGACATGCCAAATCAGCCGAAAAC
R966D rev, Tm=64°C	TATGAAGCGTAATGATGCCTTCGCTTTGGG
W1046S for, Tm= 59°C	CAAAAGACGTGTCGGCTGGGTAACTCG
W1046S rev, Tm= 61°C	CGTATCGGCCTTTTTCTTCTAGCTGC

Target mutations were introduced by inverse PCR using Eppendorf Mastercycler Gradient thermal cyclers, with buffers and enzymes supplied in the Phusion DNA Polymerase kit (NEB). Reactions (50 µL) were carried out in thin-walled 200 µL PCR tubes following manufacturer instructions. Next, template DNA was removed by a 2 h digestion with *Dpn*I followed by PCR purification (QIAquick PCR Purification Kit). Ligation reactions were performed for 1 h at 25°C with T4 DNA ligase and polynucleotide kinase. NEB 5-alpha competent *E.coli* (high efficiency) were then transformed according to manufacturer instruction, single colonies picked and grown in 5 mL Luria-Bertani medium (LB) containing 50 µg/mL kanamycin for 16 h, plasmid DNA isolated using a mini-prep kit (Qiagen) and sequence verified by plasmid sequencing.

Protein production and purification

Chemically competent *E. coli* BL21 (DE3) were transformed by heat shock with a pET28a vector (or pET28b for *Te*SADH W110A) encoding the desired *N*-terminal polyhistidine tagged enzyme under a T7 promoter. Transformants were grown on LB agar with 50 µg/ml kanamycin at 37°C for 16 hours. Starter cultures were prepared in LB medium with 50 µg/ml kanamycin at 37°C for 16 hours by picking single colonies from agar plates.

P450

Expression cultures (800 mL, TB-AIM) were inoculated with 8 mL starter culture and cells grown at 37°C 200 rpm until OD_{600}= 0.8 was reached. At this stage, 5-Aminolevulinic acid hydrochloride (5-ALA, 0.5 mM) was added and the growth continued at 20°C. After 20 h, cells were harvested by centrifugation (2500 *g*, 20 min, 4 °C) and kept at -20 °C until further use.

For protein purification, cells were resuspended in 90% buffer A (Tris-HCl 0.1 M, 0.3 M NaCl, pH 8) and 10% buffer B (Tris-HCl 0.1 M, 0.3 M NaCl, 0.3 M imidazole, pH 8) to a final concentration of 200 mg/mL cell wet weight. Cells were lysed by ultrasonication with a Bandelin Sonopuls sonicator (20 cycles, 15 s on and 45 s off, 40 % amplitude) and the supernatant obtained by ultracentrifugation (48384 *g*, 30 min, 4 °C). Filtration of the supernatant was performed with a 0.45 µm filter before loading onto a pre-equilibrated 5 mL HisTrap FF column (GE Healthcare). The target protein was purified using an ÄKTA Pure system (GE Healthcare) operated at 2 mL min^{-1} following a series of step reported in the table below:

Step	% B	Column volumes
Wash 1	10	5
Wash 2	20	5
Elution	100	10
Re-equilibration	0	5

Protein elution was followed at 280 nm, fractions collected and desalted in 0.2 M Tris-HCl, pH 8 with a 30,000 molecular weight cut-off filter (Vivaspin column, GE Healthcare). Protein concentration was either determined by CO-difference spectroscopy (P450) following the method of Omura and Sato[1] or spectrofotometrically (*Te*SADH W110A: ε=25600 M^{-1} cm^{-1}; *Cbo*FDH ε=51465 M^{-1} cm^{-1}; *Asp*RedAm ε= 24410 M^{-1} cm^{-1} , wavelength λ=280 nm). Typically, proteins were stored at -80°C as stocks at 10 mg mL^{-1} protein concentration.

When crude enzyme preparations were used to carry out biotransformation, cells were resuspended in 0.2 M Tris-HCl pH 8 to 200 mg mL^{-1} cell wet weight and lysed as described above. The supernatant obtained after ultracentrifugation was either used without further purification or concentrated until the desired protein concentration/enzymatic activity was attained.

***Te*SADH W110A and *Cbo*FDH**

Expression cultures (400 mL, TB-AIM) were inoculated with 4 mL starter culture and cells grown at 37°C 250 rpm for 24 h. Cells harvesting, storage and purification were performed as reported above.

For lyophilization of *Te*SADH W110A cell free extracts (CFE), cells were resuspended in 50 mM potassium phosphate buffer, 0.5 M NaCl, pH 7.5 to a final concentration of 300 mg/mL cell wet weight and lysed by ultrasonication as described above. The supernatant obtained by ultracentrifugation was frozen in liquid nitrogen before freeze-drying for two days (Heto Powerdry LL 1500, Thermo Electron Corporation).

***Asp*RedAm**

Expression cultures (600 mL, 2-YT broth) were inoculated with 6 mL starter culture and cells grown at 37°C 250 rpm until OD_{600}= 0.8 was reached. At this stage, isopropyl β-D-1-thiogalactopyranoside (IPTG, 0.4 mM) was added to induce protein expression and the growth continued at 20°C. Cells harvesting, storage and purification were performed as reported above.

Determination of volumetric enzymatic activities

Volumetric activities were determined by monitoring NAD(P)H formation using a microtitre plate reader (Infinite M200 Pro, Tecan, Männedorf, Switzerland) under the following conditions: Tris-HCl 0.2 M, pH 8, 1 mM NAD^+ (*Cbo*FDH) or $NADP^+$ (*Te*SADH W110A), 250 mM formate (*Cbo*FDH) or 30 mM cyclohexanol (final 4% v/v DMSO for *Te*SADH W110A), 20 µL CFE, 200 µL final volume, 22°C. Formation of the reduced cofactor was followed for 1 minute, and the volumetric units (U mL^{-1}, defined as the quantity of enzyme that reduces 1 µmol of oxidized cofactor in 1 min) were calculated from slopes of NAD(P)H formation calculated over 10-30 s using ε_{NADH} = 6.22 mM^{-1} cm-1 and a pathlength of 0.55 cm.

Chemical synthesis and characterisation

Synthetic procedures

Compounds **1a-b** were purchased from commercial suppliers. Preparation and spectroscopic characterisation for compounds **1c-f** are reported in Aleku *et* al.[2]

Reductive amination procedure for the preparation of amine products 4c-d and 5c

To a solution of the corresponding ketone (2.0 mmol) in dry THF (5 mL) under N_2 were added the corresponding amine (2.2 mmol), sodium triacetoxyborohydride (0.636 g, 3.0 mmol) and glacial acetic acid (0.114 mL, 2.0 mmol). The reaction was stirred for 16 hours at 20°C under N_2 then carefully quenched by addition of 1 M aqueous HCl (10 mL). Ethyl acetate (EtOAc, 10 mL) was added and the phases separated. The aqueous phase was extracted with a further portion of EtOAc (10 mL). The aqueous phase was then basified to pH 12 by addition of 5 M NaOH. The product was extracted into EtOAc (2 x 20 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to afford the corresponding amine products with no further purification required.

Reductive amination procedure for the preparation of amine products 4f

To a stirred flask of cyclopentanone (0.2 g, 2.4 mmol) in dry methanol (MeOH, 10 mL) over 4 Å molecular seives under nitrogen was added cyclopropylamine (0.332 mL, 4.8 mmol). The reaction was stirred at room temperature overnight, after which $NaBH_4$ (0.181 g, 4.8 mmol) was added over 10 minutes. The mixture was stirrer for a further 2 hours, then the solvent was removed under reduced pressure. The resulting slurry was resuspended in EtOAc (20 mL), filtered then extracted twice with 1 M HCl. The aqueous phase was then basified (pH 12) with 5 M NaOH and extracted three times with EtOAc. The organic phases were combined, dried over $MgSO_4$ and the solvent removed under reduced pressure to afford the title compound as a yellow oil.

Typical procedure for RedAm-catalysed reductive amination

A 500 µL reaction mixture contained 20 mM D-glucose, 0.5 mg mL^{-1} GDH (Codexis, CDX-901), 0.5 mM $NADP^+$, 1 mg mL^{-1} purified RedAm, 5 mM ketone, 100 mM amine nucleophile (in Tris-HCl buffer adjusted to pH 9) and 2 % v/v DMSO. Reactions were incubated at 30 °C with 250 rpm shaking for 24 h, after which they were quenched by the addition of 30 µL of 10M NaOH and extracted twice with 500 µL methyl *tert*-butyl ether (MTBE). The organic fractions were combined and dried over anhydrous $MgSO_4$ and analysed by GC-FID.

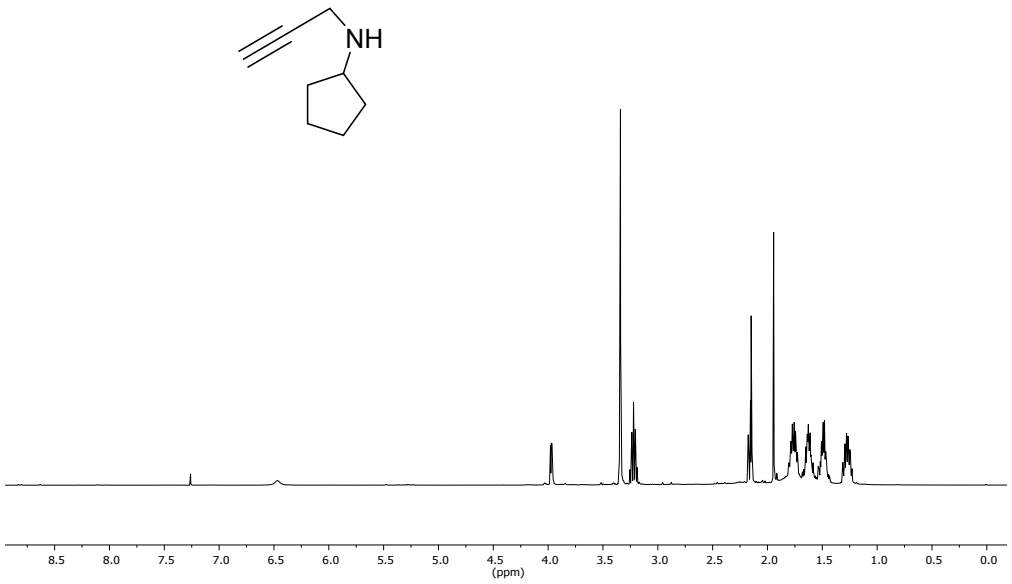
Spectroscopical characterisation

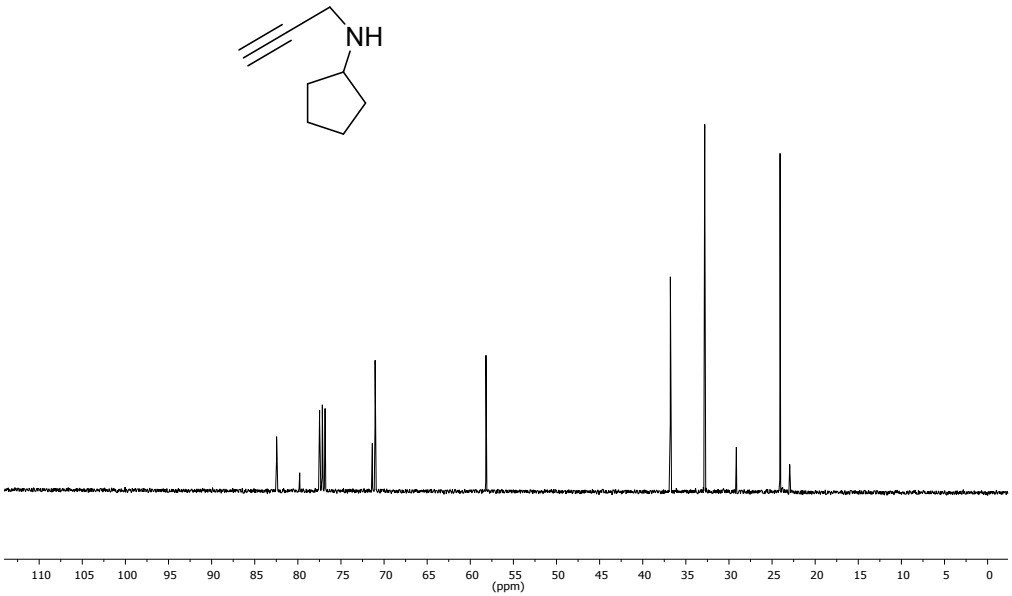
Spectra from ^{1}H and ^{13}C NMR runs were recorded on a Bruker Avance 400 instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C) in $CDCl_3$ using residual protic solvent as an internal standard. Reported chemical shifts (δ) (in parts per million (ppm)) are relative to the residual protic solvent signal ($CHCl_3$ in $CDCl_3$, 1H = 7.26; 13C = 77.0).

High-resolution mass spectrometry (HRMS) was recorded using a Waters LCT time-of-flight mass spectrometer, connected to a Waters Alliance LC (Waters, Milford, MA, USA). Data were processed with Waters Masslynx software.

N-propargylcyclopentylamine 4c

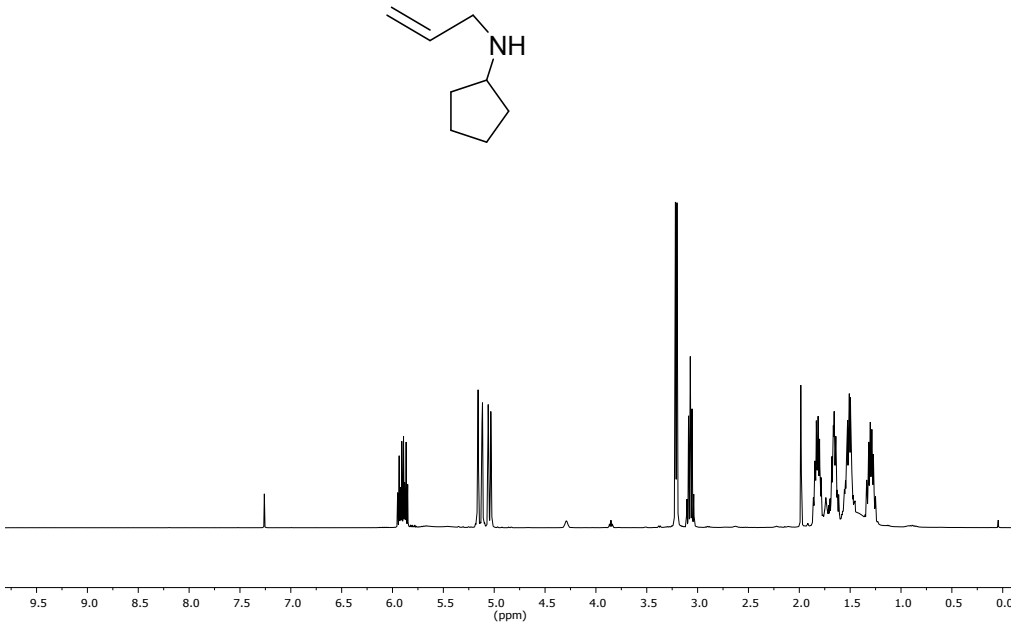
Brown oil isolated, 12% yield. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 3.41 (d, *J* = 2.5 Hz, 2H), 3.29 (quint, *J* = 11.2 Hz, 1H), 2.20 (t, *J* = 2.5 Hz, 1H), 1.80 – 1.44 (m, 9H). **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 82.46 (C), 70.98 (CH), 58.18 (CH), 36.79 (CH_2), 32.80 (CH_2), 24.06 (CH_2). **HRMS** calcd. for $C_8H_{14}N^+$ 124.1121 $[M+H]^+$, found 124.1099.

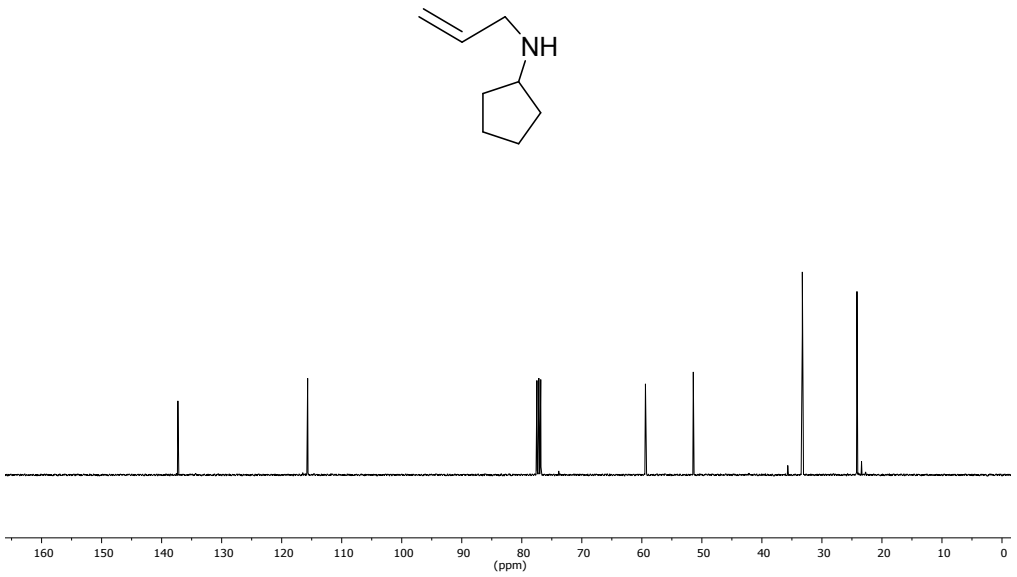




N-allylcyclopentylamine 4e

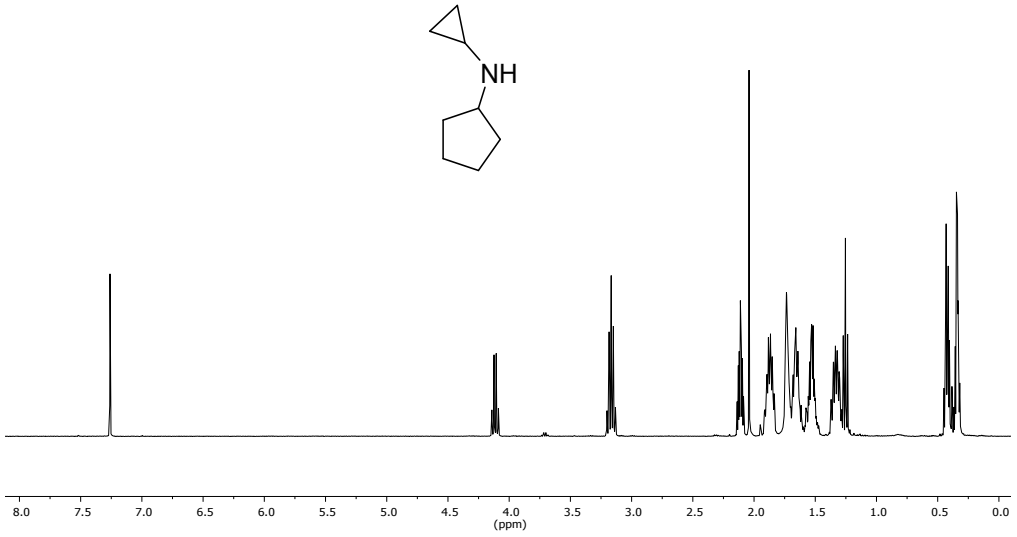
Yellow oil isolated, 58% yield. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 6.01 – 5.70 (m, 1H), 5.27 – 4.96 (m, 2H), 4.07 – 3.65 (m, 1H), 3.22 (dt, *J* = 6.1, 1.4 Hz, 1H), 3.08 (p, *J* = 6.8 Hz, 1H), 1.92 – 1.73 (m, 2H), 1.77 – 1.57 (m, 2H), 1.59 – 1.44 (m, 2H), 1.31 (ddd, *J* = 13.3, 7.6, 4.0 Hz, 2H).. **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 137.3 (CH), 115.7 (CH_2), 59.4 (CH), 51.4 (CH_2), 42.2 (CH_2), 33.3 (CH_2), 24.2 (CH_2), 23.4 (CH_2). **HRMS** calcd. for $C_8H_{16}N^+$ 126.1277 $[M+H]^+$, found 126.1265.

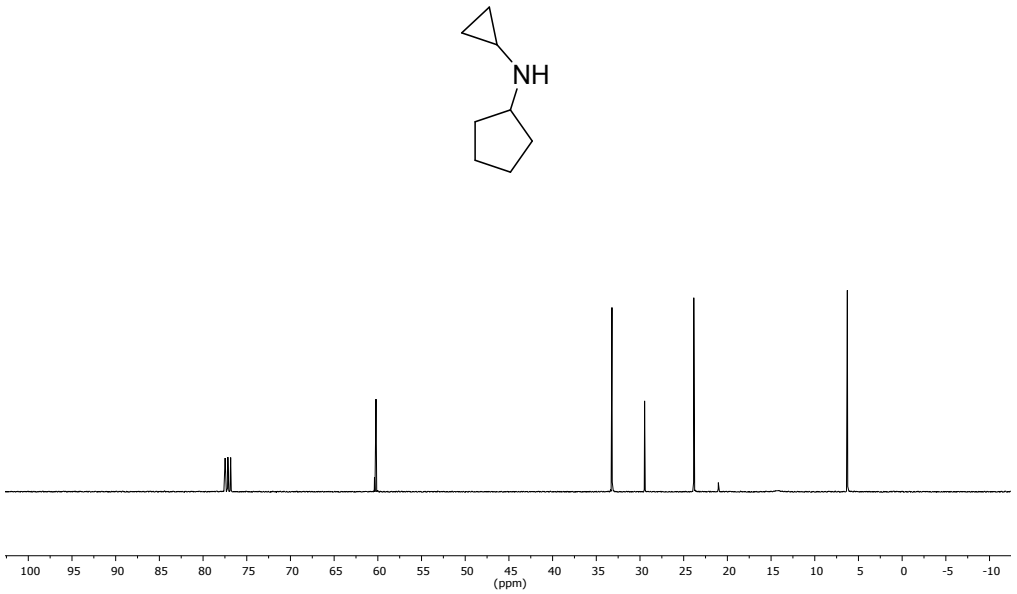




N-cyclopropylcyclopentylamine 4f

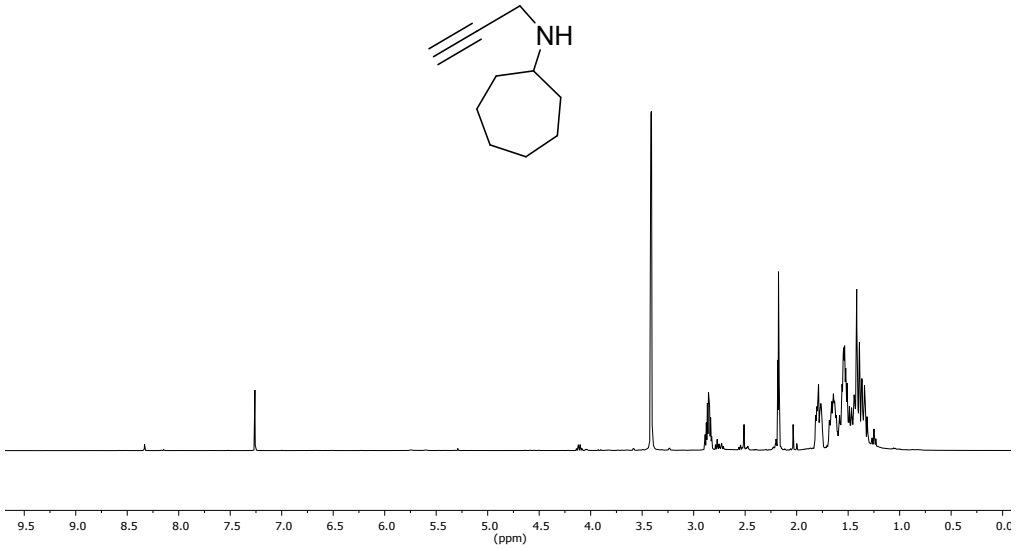
Yellow oil isolated, 64% yield. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 3.02 (quin, *J* = 6.9 Hz, 1H), 1.99 – 1.94 (m, 1H), 1.76 – 1.69 (m, 2H), 1.61 – 1.56 (s, 1H), 1.55 – 1.45 (m, H), 1.43 – 1.33 (m, 2H), 1.23 – 1.15 (m, 2H), 0.32 – 0.17 (m, 4H). **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 60.13 (CH), 33.12 (CH_2), 29.39 (CH), 23.76 (CH_2), 6.16 (CH_2). **HRMS** calcd. for $C_8H_{16}N^+$ 126.1204 $[M+H]^+$, found 126.1281.

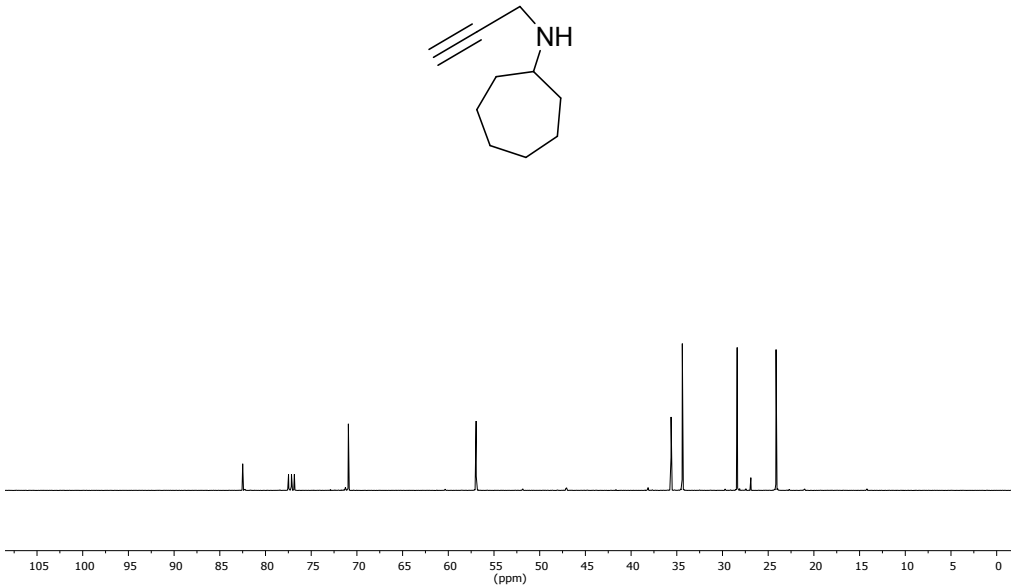




N-propargylcycloheptylamine 5c

Orange oil isolated, 76% yield. **^{1}H NMR** δ_H (400 MHz, $CDCl_3$) 3.43 (d, *J* = 2.5 Hz, 2H), 2.87 (quint, *J* = 10.5, 4.1 Hz, 1H), 2.20 (t, *J* = 2.5 Hz, 1H), 1.82 – 1.79 (s, 1H), 1.67 – 1.53 (m, 12H). **^{13}C NMR** δ_C (100 MHz $CDCl_3$) 82.40 (C), 70.98 (CH), 56.97 (CH), 35.59 (CH_2), 34.35 (CH_2), 28.36 (CH_2), 24.13 (CH_2). **HRMS** calcd. for $C_{10}H_{18}N^+$ 152.1434 $[M+H]^+$, found 152.1400.





Biocatalytic cascade for the amination of cycloalkanes

Amine inhibition studies

In order to investigate the effect of amines **a–f** at different loadings on the performance of the P450, the hydroxylation step was carried out in 28 mL glass vials, with 6 μM P450, 0.3 U mL^{-1} *Cbo*FDH (both added as concentrated CFE), 1 mM NAD^+, 250 mM sodium formate, various concentrations of the amine nucleophile (as reported in Figure 1) and finally 125 μL cycloalkane added neat. Reaction volumes were made up to 500 μL with Tris-HCl 0.2 M, pH 8 and vials incubated in horizontal position at 20°C with 200 rpm orbital shaking for 24 h. Reactions were extracted in 500 μL MTBE (2 x 250 μL). with 2 mM decane as internal standard. The organic fractions were dried over anhydrous $MgSO_4$ and analyzed by GC-FID as reported in the section "Analytics". The relative performance was expressed as residual total turnover numbers (TTNs, ratio between total product concentration and P450 concentration, calculated over 24 h), that is the ratio between TTNs in the presence of the amine and TTNs when the amine nucleophile was not added to the reaction mixture.

One-step process

The one-step cascade was carried out in 28 mL glass vials, with 1.8 μM P450, 0.16 U mL^{-1} *Cbo*FDH (both added as freshly lysed CFE), 0.3 U mL^{-1} *TeS*ADH W110A (added as rehydrated lyophilized CFE), 2 mg mL^{-1} purified *Asp*RedAm, 1 mM of both NAD^+ and $NADP^+$, 250 mM sodium formate, various concentrations of the amine nucleophile (as reported in Table S1) and finally 125 μL cyclohexane added neat.

When purified proteins were employed, reactions were set-up similarly, with 6 μM P450, 1 U mL^{-1} *Cbo*FDH, 2 U mL^{-1} *TeS*ADH W110A, 2 mg mL^{-1} *Asp*RedAm and 25 mM allylamine. The amount of cyclohexane added was reduced to 20 μL in order to decrease possible inhibitory effects on enzymatic performance while preserving P450 activity.[3]

Reaction volumes were made up to 500 μL with Tris-HCl 0.2 M, pH 8 and vials incubated in horizontal position at 20°C with 200 rpm orbital shaking for 24 h. Reactions were quenched by addition of NaOH (5 M, 100 μL) and extracted with 500 μL MTBE (2 x 250 μL) with 2 mM decane as internal standard. The organic fractions were dried over anhydrous $MgSO_4$ and analyzed by GC-FID as reported in the section "Analytics". When ammonia was employed as amine nucleophile, samples were derivatized with acetic anhydride and an excess of triethylamine at room temperature prior to analysis.

Two-step process

The two-step biocatalytic cascade was carried out in 28 mL glass vials, with 6 μM P450, 0.3 U mL^{-1} *Cbo*FDH (both added as concentrated CFE), 1 mM NAD^+, 250 mM sodium formate and finally 125 μL cycloalkane added neat. Reaction volumes were made up to 500 μL with Tris-HCl 0.2 M, pH 8 and vials incubated in horizontal position at 20°C with 200 rpm orbital shaking for 24 h. Afterwards, the components for the second step were added as follows: 0.2 U mL^{-1} *TeS*ADH W110A (added as rehydrated lyophilized CFE), 2 mg mL^{-1} purified *Asp*RedAm, 1 mM $NADP^+$ and 100 mM of the appropriate amine nucleophile. Reaction volumes were made up to 750 μL and vials incubated at 30°C with 200 rpm shaking.

When purified proteins were used, reactions were set-up employing the same amount of enzyme/enzymatic activity as described above. Similarly to the one-step process with purified proteins, 20 μL cyclohexane and 25 mM allylamine were employed.

After 24 h, reactions were quenched by addition of NaOH (5 M, 150 μL) and extracted with 750 μL MTBE (2 x 375 μL) with 2 mM decane as internal standard. When cyclopentane was used as starting material, 4 mM cyclohexane instead of 2 mM decane was used as internal standard. The organic fractions were dried over anhydrous $MgSO_4$ and analyzed by GC-FID as reported in the section “Analytics”. When ammonia was employed as amine nucleophile, samples were derivatized with acetic anhydride and an excess of triethylamine at room temperature.

Preparative-scale production of *N*-propargylcyclohexylamine 1c by two-step amination cascade with cyclohexane and propargylamine c

The two-step amination cascade was scaled-up linearly to a final volume of 30 mL. The first step was carried out on a 20 mL scale using Tris-HCl 0.2 M, pH 8 containing 6 μM P450, 0.3 U mL^{-1} *Cbo*FDH (both added as concentrated CFE), 1 mM NAD^+, 250 mM sodium formate and finally 5 mL cyclohexane added neat. Reactions were performed either employing a 500 mL round-bottom flask with magnetic stirring (200 rpm) or a 250 mL conical flask incubated at 18°C with 150 rpm orbital shaking. The latter proved to be superior in terms of cyclohexanol **2** produced after 19 h (Figure S3). Similarly to small-scale reactions, the second step was performed with 0.2 U mL^{-1} *TeS*ADH W110A (added as rehydrated lyophilized CFE), 2 mg mL^{-1} purified *Asp*RedAm, 1 mM $NADP^+$ and 100 mM propargylamine **c**. The reaction was incubated at 30°C and 150 rpm orbital shaking for 2 hours. The solution was then basified with 5 M NaOH (1 mL) and the product extracted into MTBE (2 x 30 mL). The organics were separated by centrifugation (4°C, 2,831 *g*, 2 minutes), collected, dried over anhydrous $MgSO_4$ and the solvent removed under reduced pressure to give a yellow oil. The latter was dissolved in 10 mL dry diethyl and the product (50 mg) was obtained as yellow hydrochloride salt after addition of 2 M ethereal HCl (300 μL) and filtration. Further details can be found in the section “GC-FID chromatograms”.

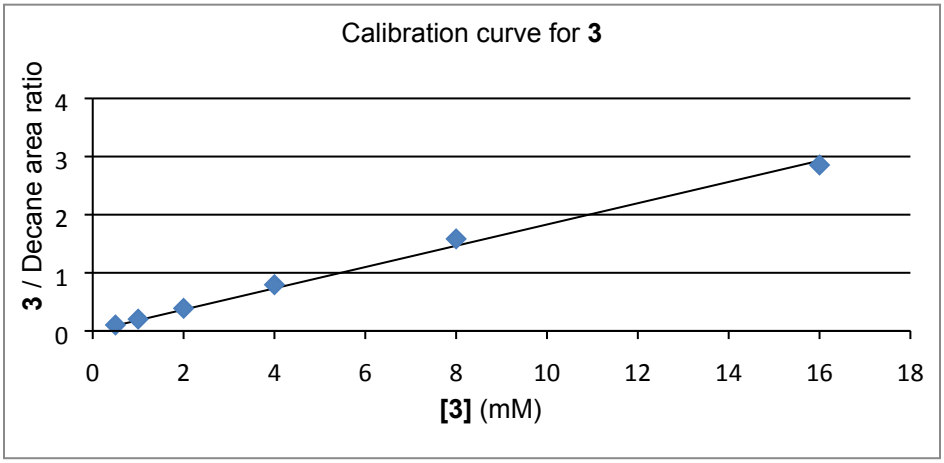
NMR and HRMS characterisation data are in accordance with previously reported data.[2,4]

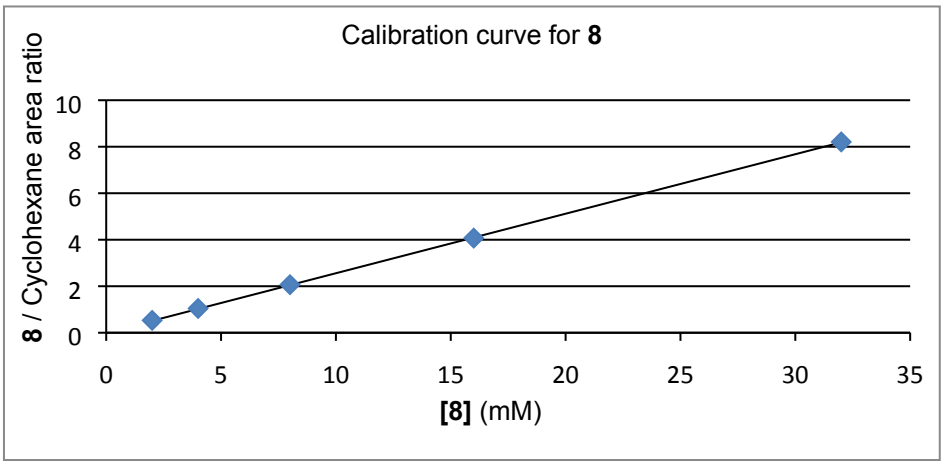
Analytics

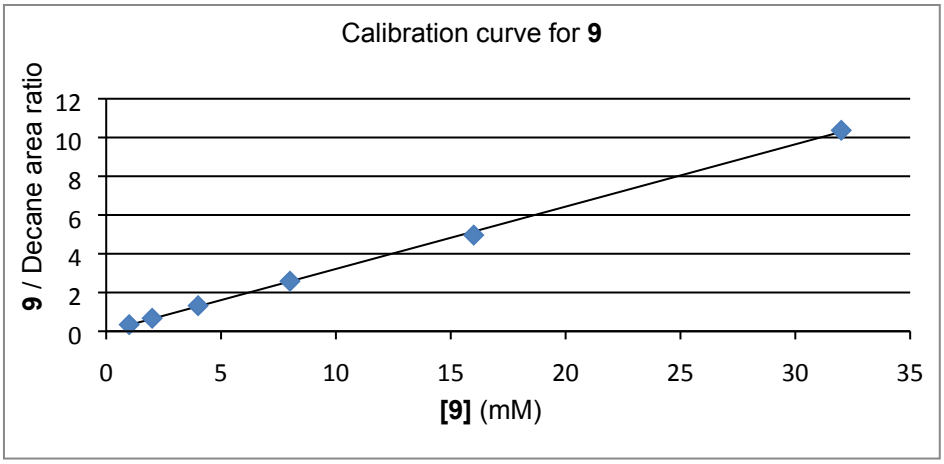
GC analysis was performed on an Agilent 6850 system (Agilent, Santa Clara, CA, USA) with a flame ionization detector (FID) equipped with an CP-ChiraSil-DEX CB column (Agilent, Santa Clara, CA, USA) with 0.25 µm film thickness and 0.25 mm internal diameter. After sample preparation, 2 µL were injected at a split ratio 100:1. The inlet temperature was set at 200°C, the detector temperature at 250°C and the pressure maintained at 6.8 psi, using helium as carrier gas. The following method was applied: 50°C; 10°C min^{-1} to 200°C; hold 2 min.

Yields were determined using the appropriate calibration curve internal standard *vs* cyclopentanone **8**, cyclohexanone **3** and cycloheptanone **9**. Moreover, calibration curves were also constructed for *N*-propargylcyclohexylamine **1c** and *N*-cyclopropylcyclohexylamine **1f**.

GC-MS analysis was performed on a Agilent 7890B/5977B series gas chromatograph/mass selective detector equipped (electron impact positive mode) with an Agilent HP-1 MS column (0.32 mm internal diameter, 30 m length, 0.25 µm film thickness). Helium was used as the carrier gas at flow rate of 1.6 ml min^{-1} and the injection temperature was 270°C. Injection volume was 2 µl and samples were injected at a split ratio of 10:1. The following method was applied: 50°C; 10°C min^{-1} to 200°C; hold 2 min.






Calibration curve for 9
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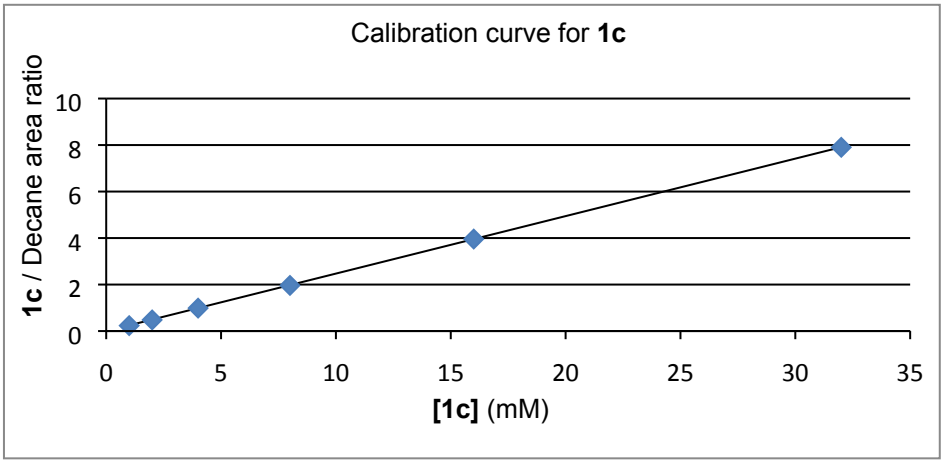
[9] (mM)


Calibration curve for 1c
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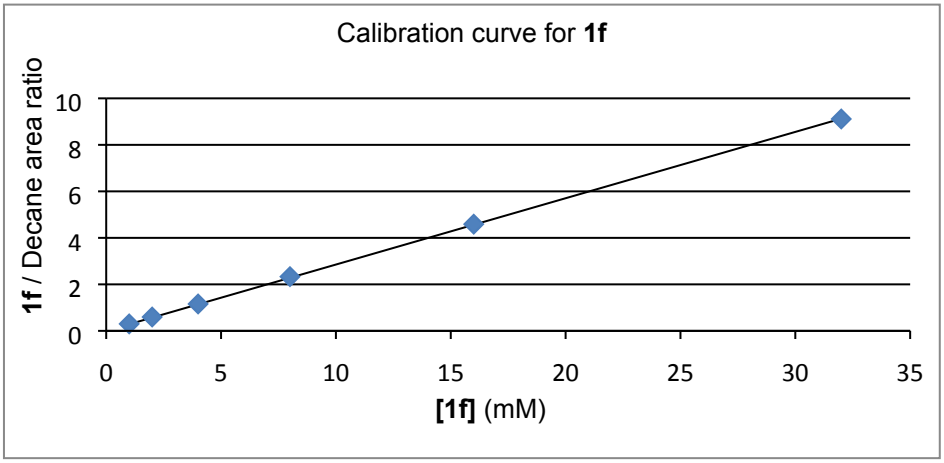
[1c] (mM)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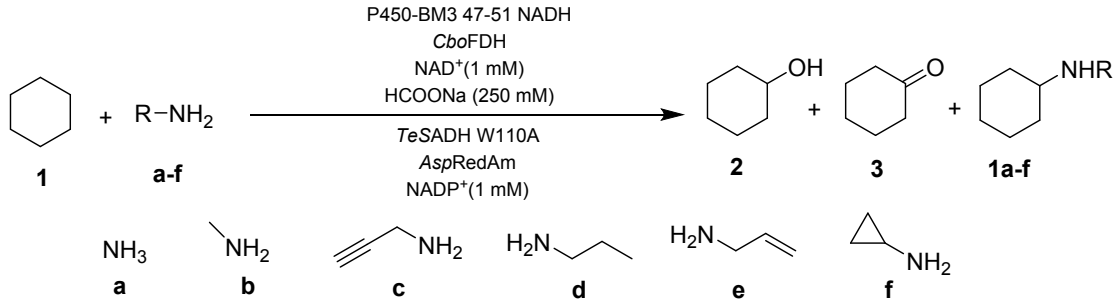
[1f] (mM)

Supporting figures and tables

Table S1. One-step amination of cyclohexane at different amine loadings. Values reported in the text and in Table S2 are written in bold.



Amine donor	Amine donor concentration (mM)	Product concentration (mM)		
		Cyclohexanol	Cyclohexanone	Amine
	250	1.5	11.6	0.2
a	**100**	**2.3**	**9.6**	**0.2**
	50	4.0	9.7	0.1
	250	1.8	5.1	4.5
b	**100**	**3.6**	**6.9**	**5.4**
	50	6.0	8.2	4.7
	25	**1.4**	**0.7**	**3.6**
c	10	4.7	2.7	4.1
	50	**6.7**	**9.3**	**5.3**
d	25	6.9	9.5	2.5
	10	15.2	14.2	1.8
	50	1.9	3.4	6.1
e	**25**	**4.7**	**5.9**	**8.3**
	10	9.4	9.3	6.2
	50	0.2	0.2	1.7
f	**25**	**0.5**	**0.7**	**2.1**
	10	1.0	1.9	1.6

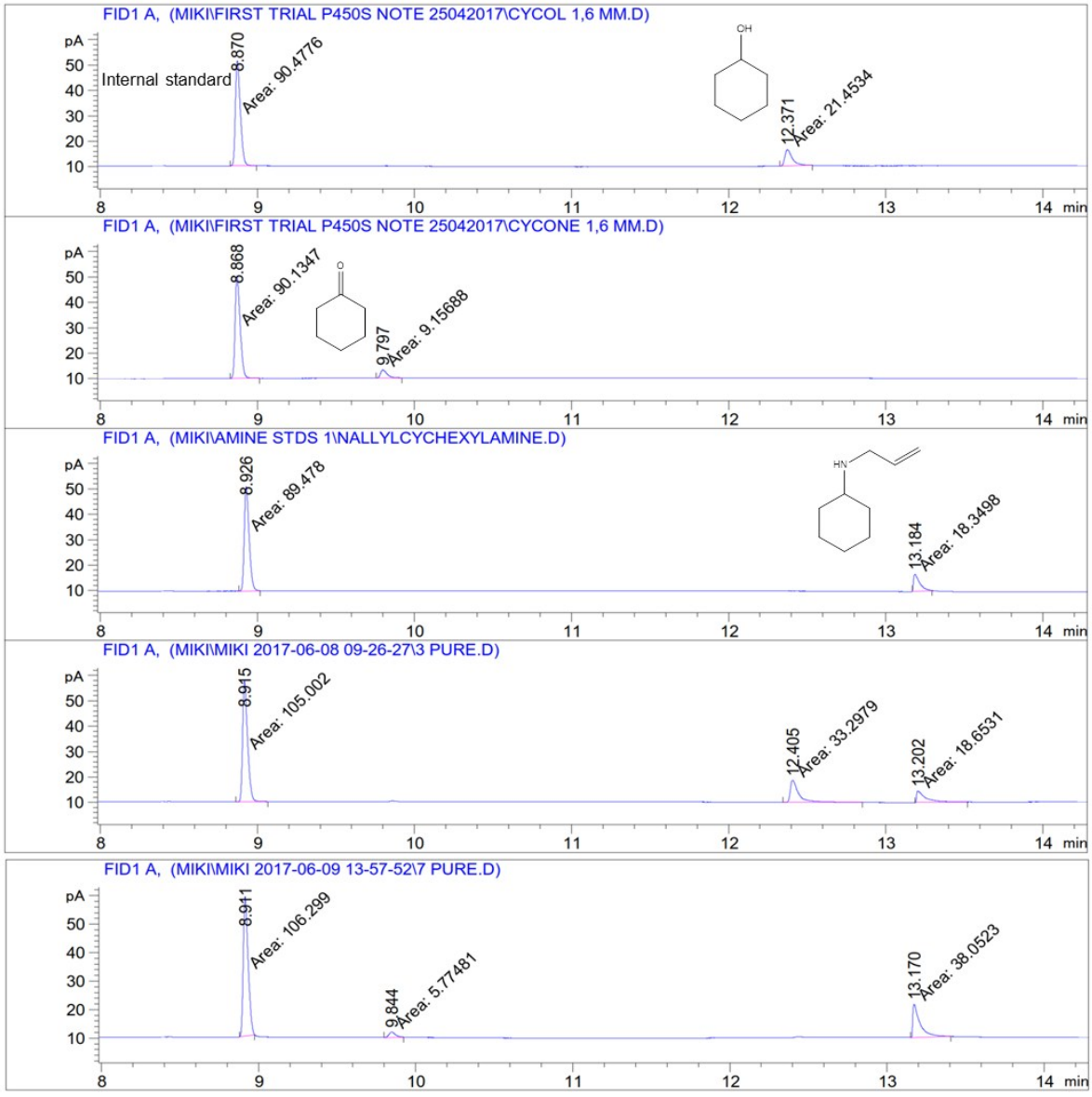
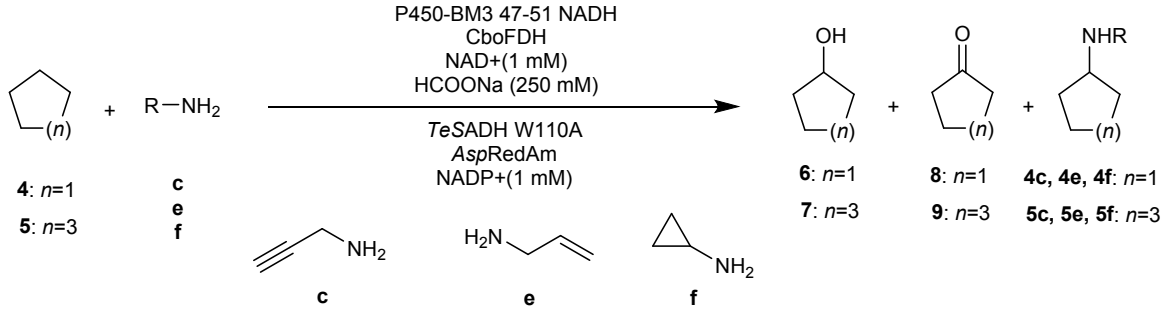


Figure S1. GC-FID chromatogram for the one-pot cascade for the amination of cyclohexane **1** with allylamine **e** employing purified proteins. Standards (top, second and third trace), one-step process (fourth trace) and two-step process (fifth trace) are shown. Yields of 0.6 mM amine **1e** were achieved when the reaction was carried out in one-step, even if a substantial amount of cyclohexanol **2** remained unreacted. Yields of 1.2 mM amine **1e** can be calculated for the two-step process with only trace amount of cyclohexanone **3** detected. Moreover, lower ADH loadings were needed in the two-step compared to the one-step, suggesting again that the P450 mediated hydroxylation can disrupt the oxidation-reductive amination step (see main text). Nevertheless, the poor yield of amine product lead us to employ crude enzyme preparations, instead of purified proteins.

Table S2. Comparison between the one-step and the two-step amination of cyclohexane.

	Amine donor	Amine donor concentration (mM)	Product concentration (mM)		
			Alcohol	Ketone	Amine
One-step	**a**	100	2.3	9.6	0.2
	b	100	3.6	6.9	5.4
	c	25	1.4	0.7	3.6
	d	50	6.7	9.3	5.3
	e	25	4.7	5.9	8.3
	f	25	0.5	0.7	2.1
Two-step	**a**	100	3.4	15.2	0.4
	b	100	0.9	10.1	8.7
	c	100	2.0	5.0	19.6
	d	100	0.3	9.5	8.0
	e	100	0.7	8.7	15.4
	f	100	3.3	3.9	17.1

Table S3. Two-step amination of cyclopentane and cycloheptane with amines **c**, **e** and **f**.



Cycloalkane	Amine donor	Product concentration (mM)		
		Alcohol	Ketone	Amine
Cyclopentane	**c**	7.3	3.2	10.6
	e	7.1	5.0	2.2
	f	8.1	3.7	3.0
Cycloheptane	**c**	0.0	5.1	3.3

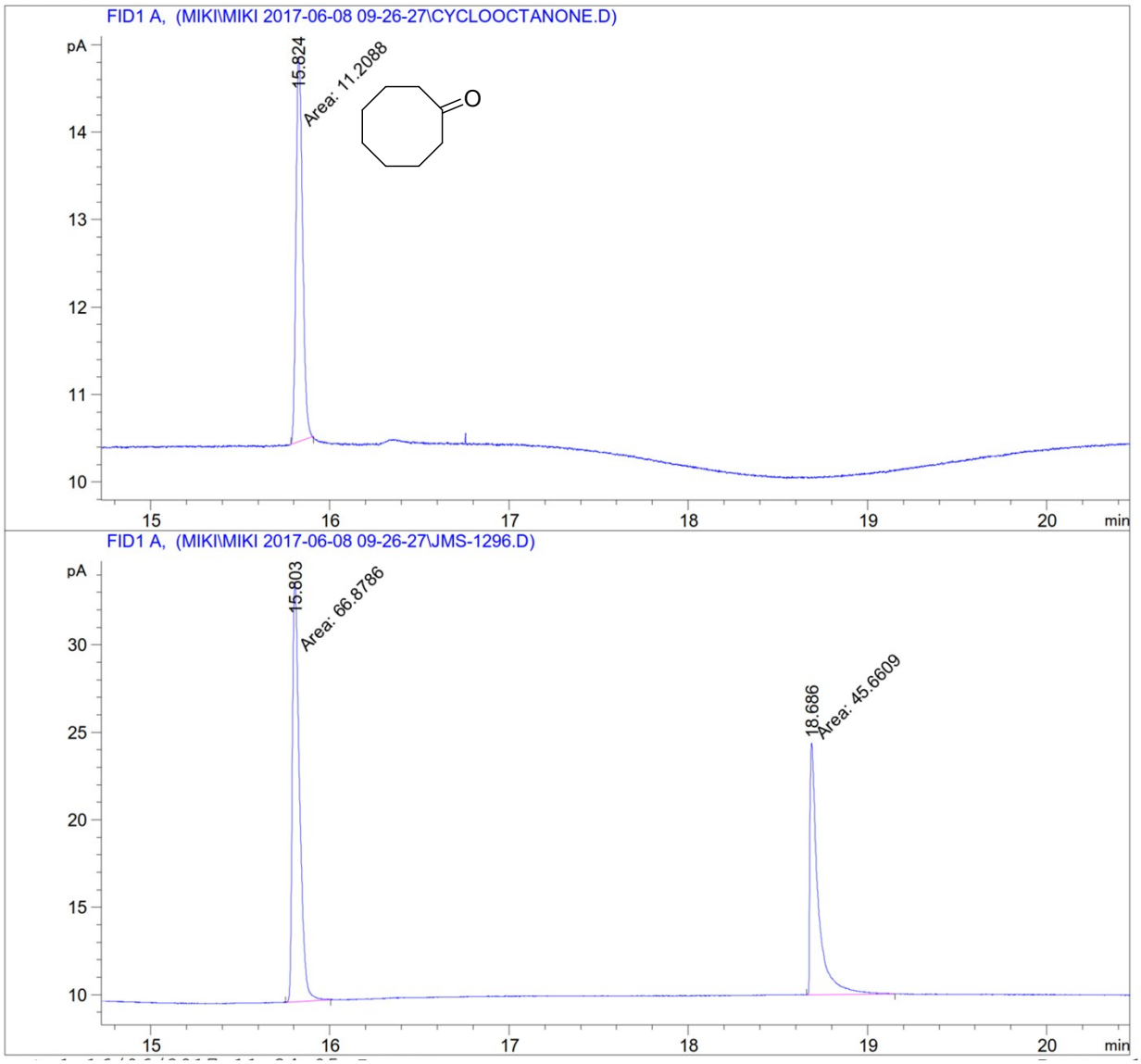


Figure S2. GC-FID chromatogram for the reductive amination of cyclooctanone with allylamine **e** employing *Asp*RedAm . Standard (top) and biotransformation (second trace) are shown. A putative product peak in the biotransformation trace (40% conversion) suggests that *Asp*RedAm is active in the reductive amination of cyclooctanone. Further details are given in the section “GC-MS traces”.

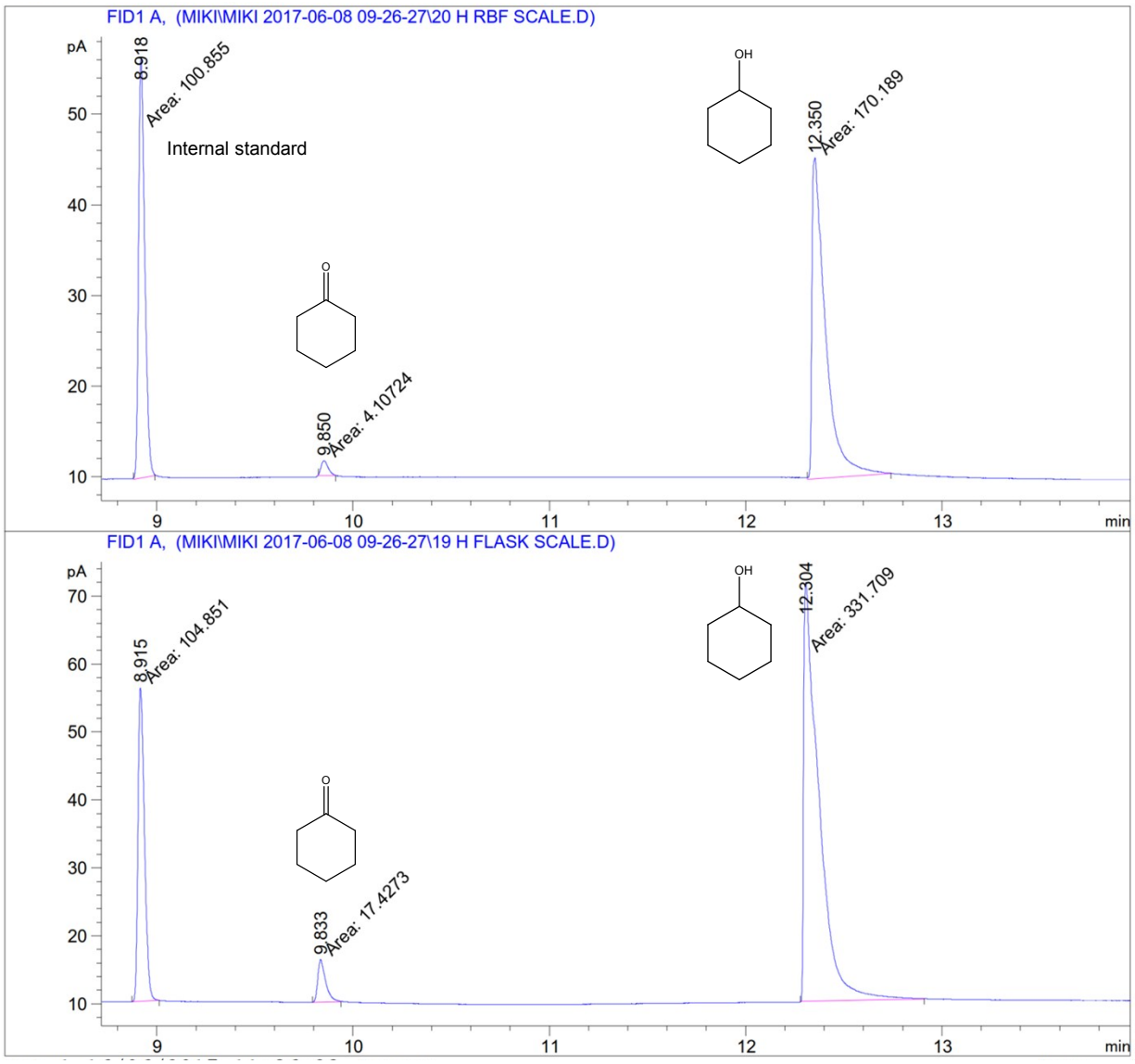


Figure S3. GC-FID chromatogram for the first step of the preparative scale amination of cyclohexane **1** showing cyclohexanol **2** production after 19-20 h. Changing the reaction vessel from a stirred round bottom flask (top trace) to a shaken conical flask (bottom trace), almost doubled the amount of cyclohexanol **2** produced after 19-20 h. Additionally, cyclohexanol **2** might be overoxidized by the P450, as suggested by cyclohexanone **3** formation.

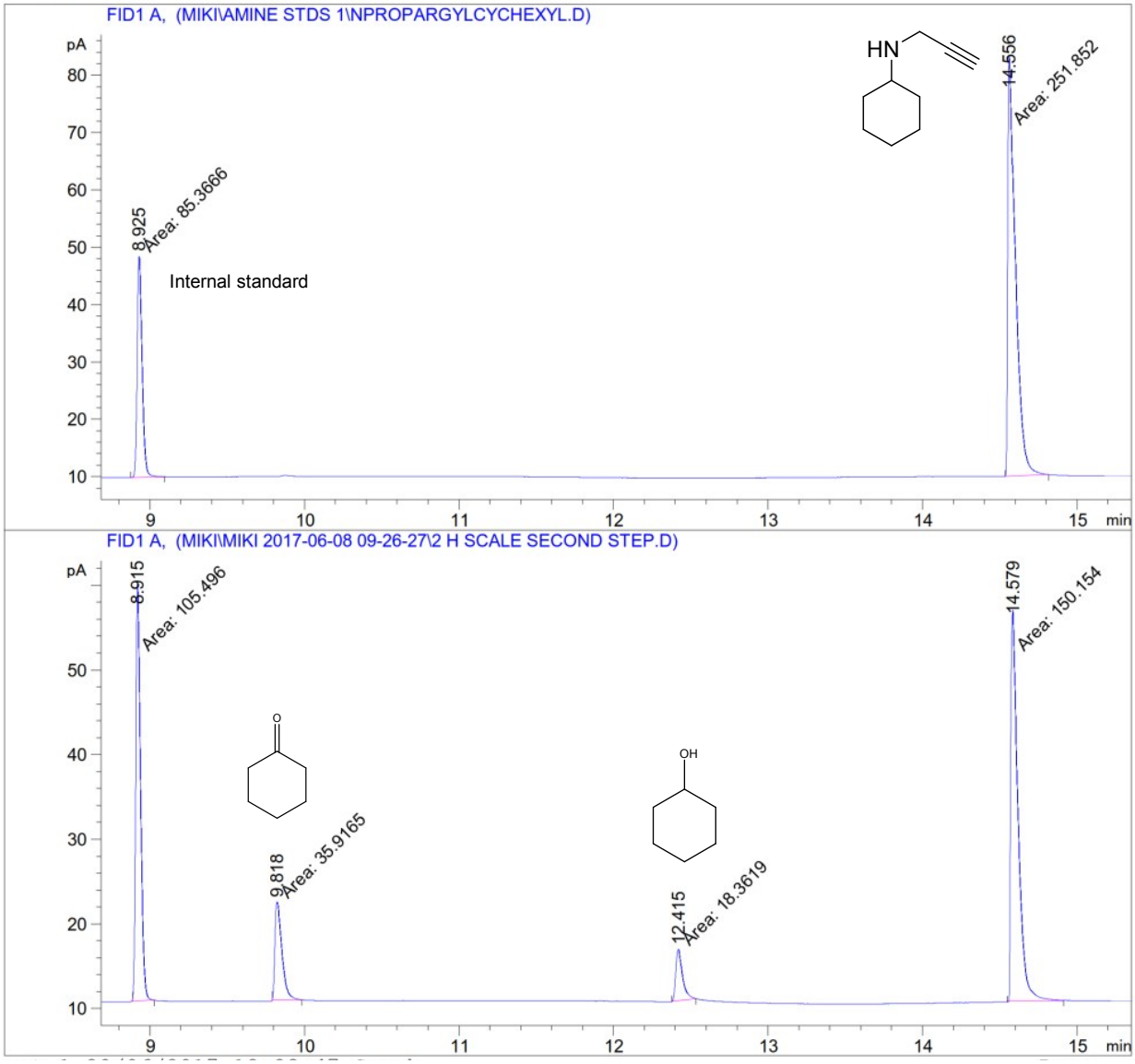
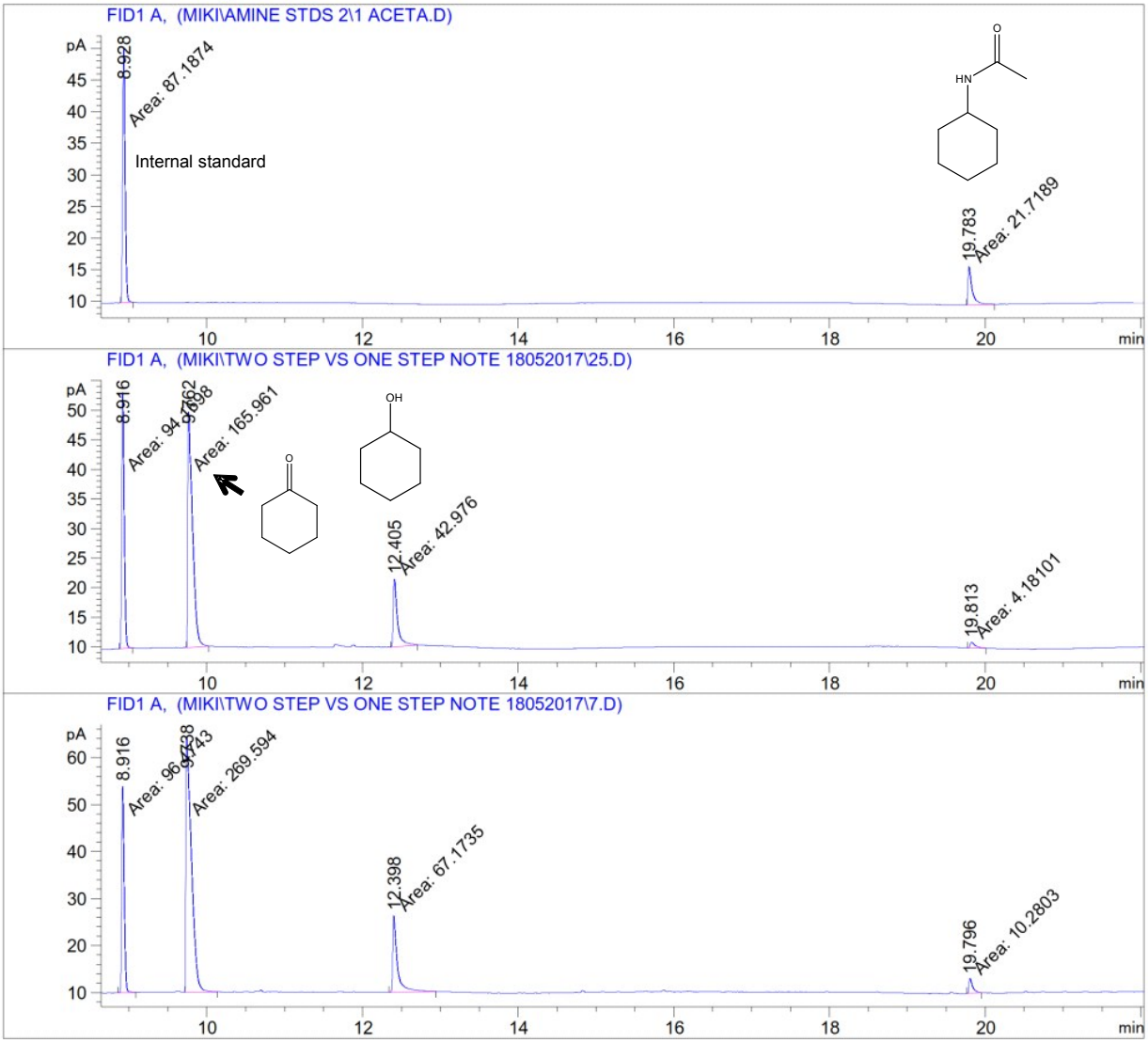


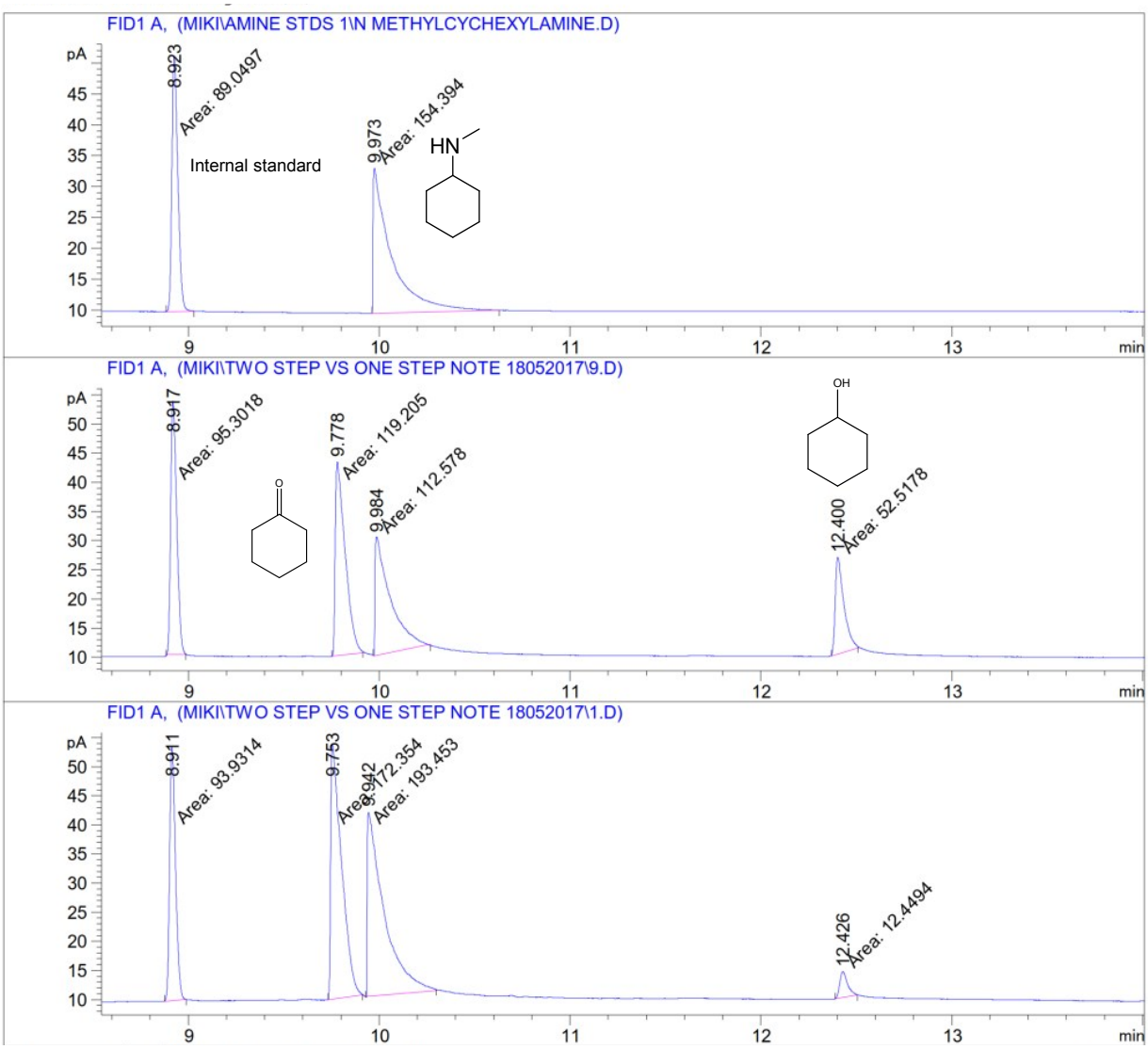
Figure S4. GC-FID chromatogram for the second step of the preparative scale amination of cyclohexane **1** with propargylamine **c** showing product standard (top trace) and biotransformation after 2 h.

GC-FID chromatograms and GC-MS traces

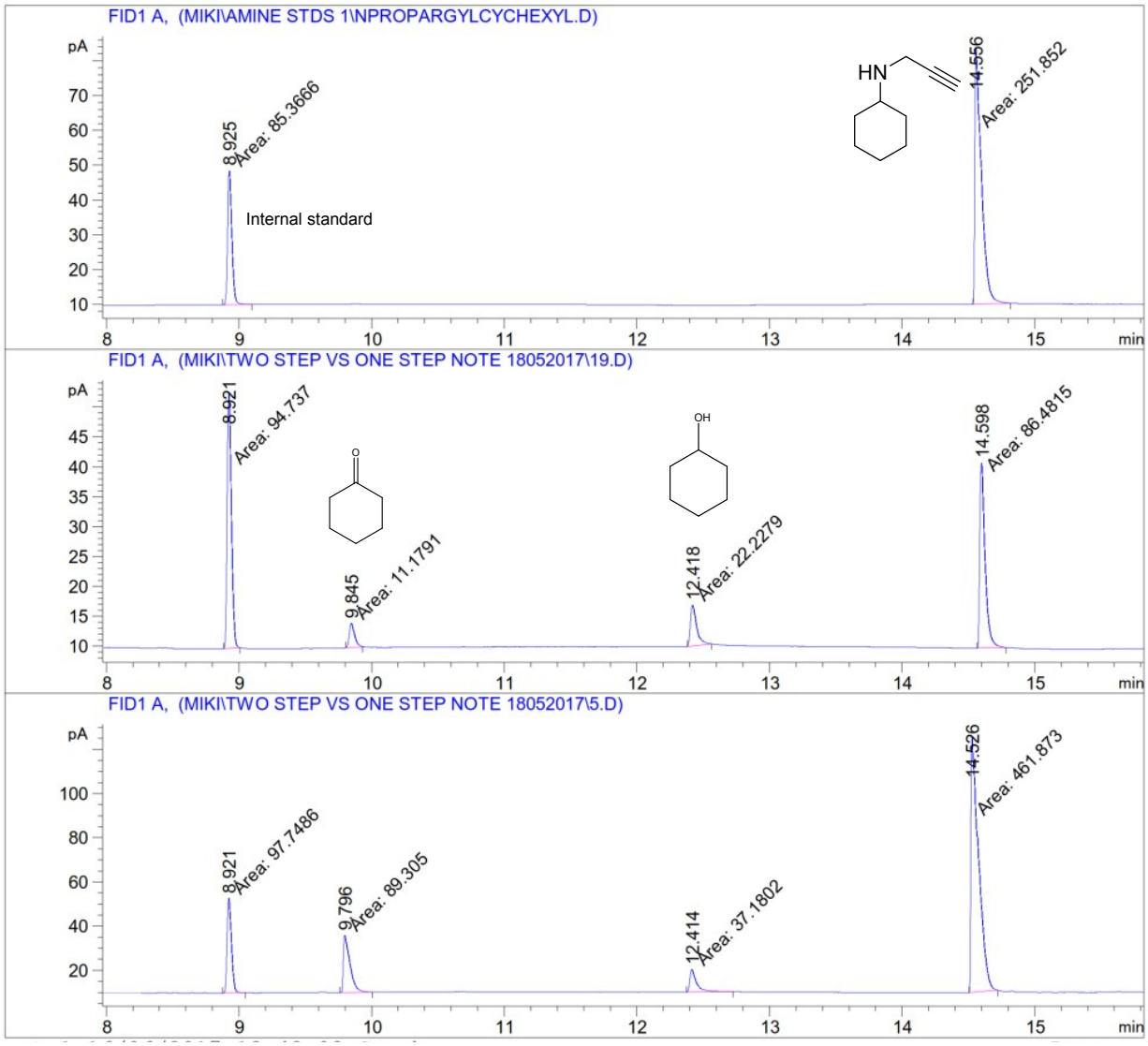
GC-FID chromatograms



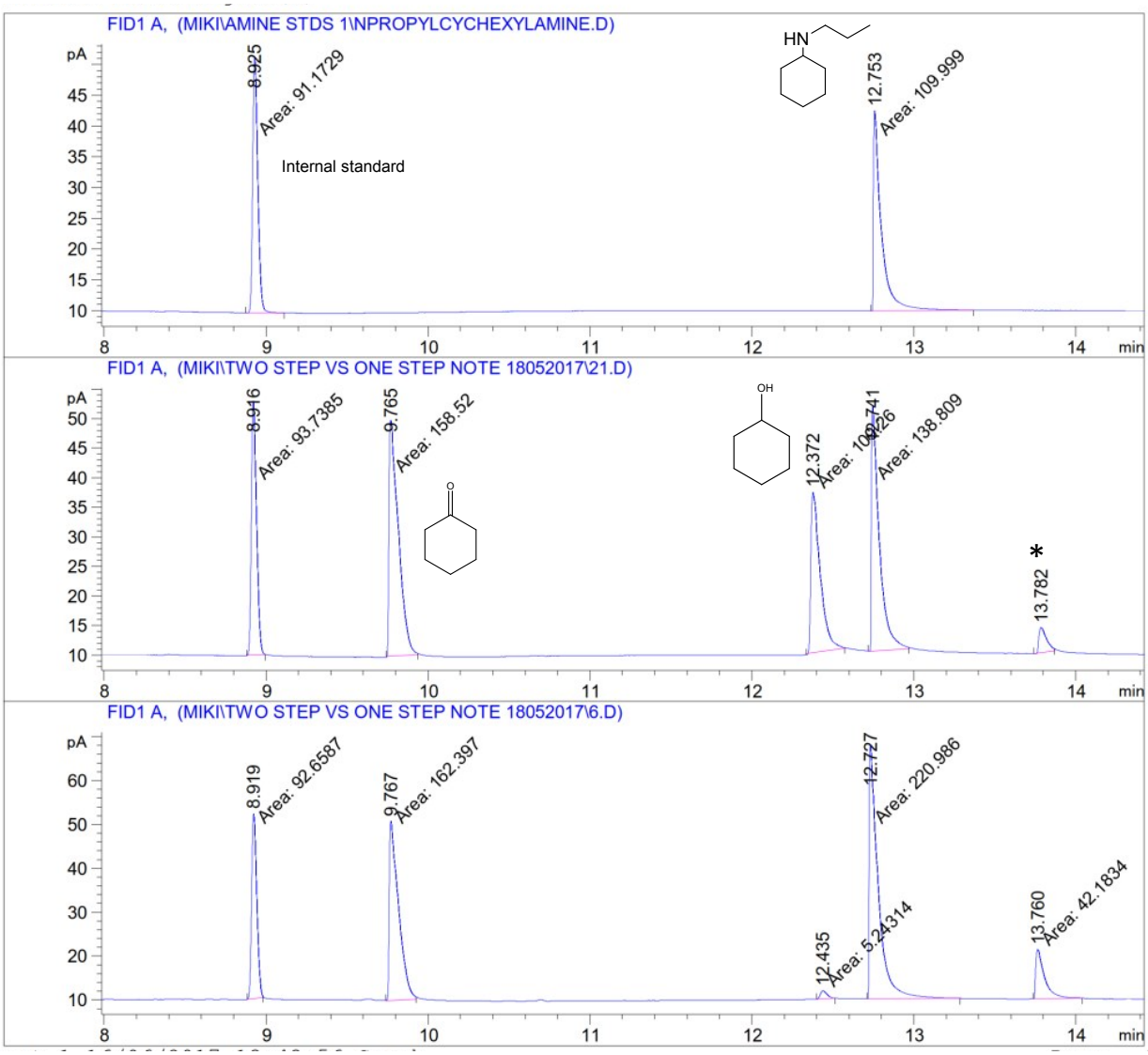
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with ammonia **a** . Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown. Samples were derivatized with acetic anhydride and an excess of triethylamine at room temperature prior to analysis.



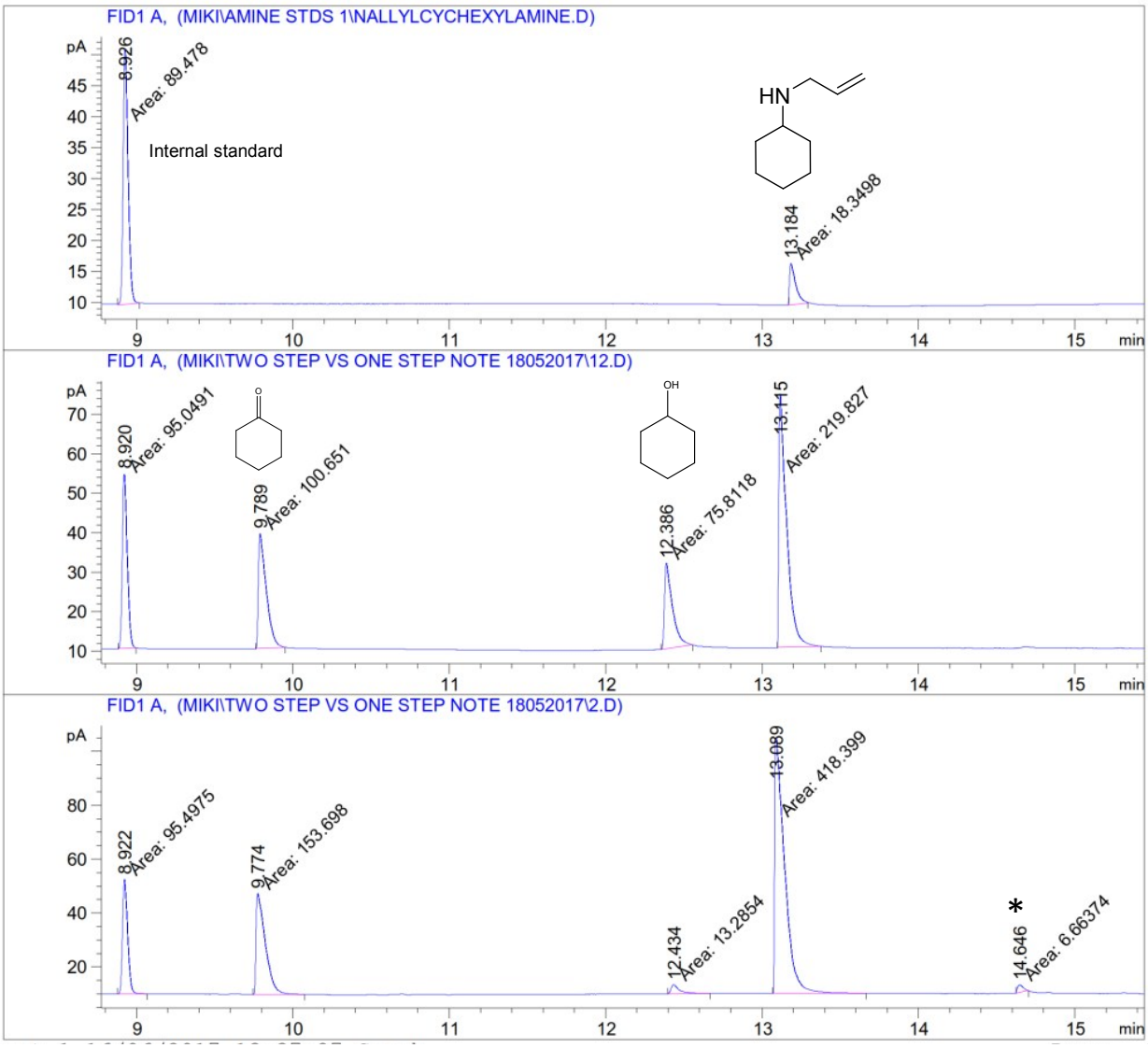
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with methylamine **b**. Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown.



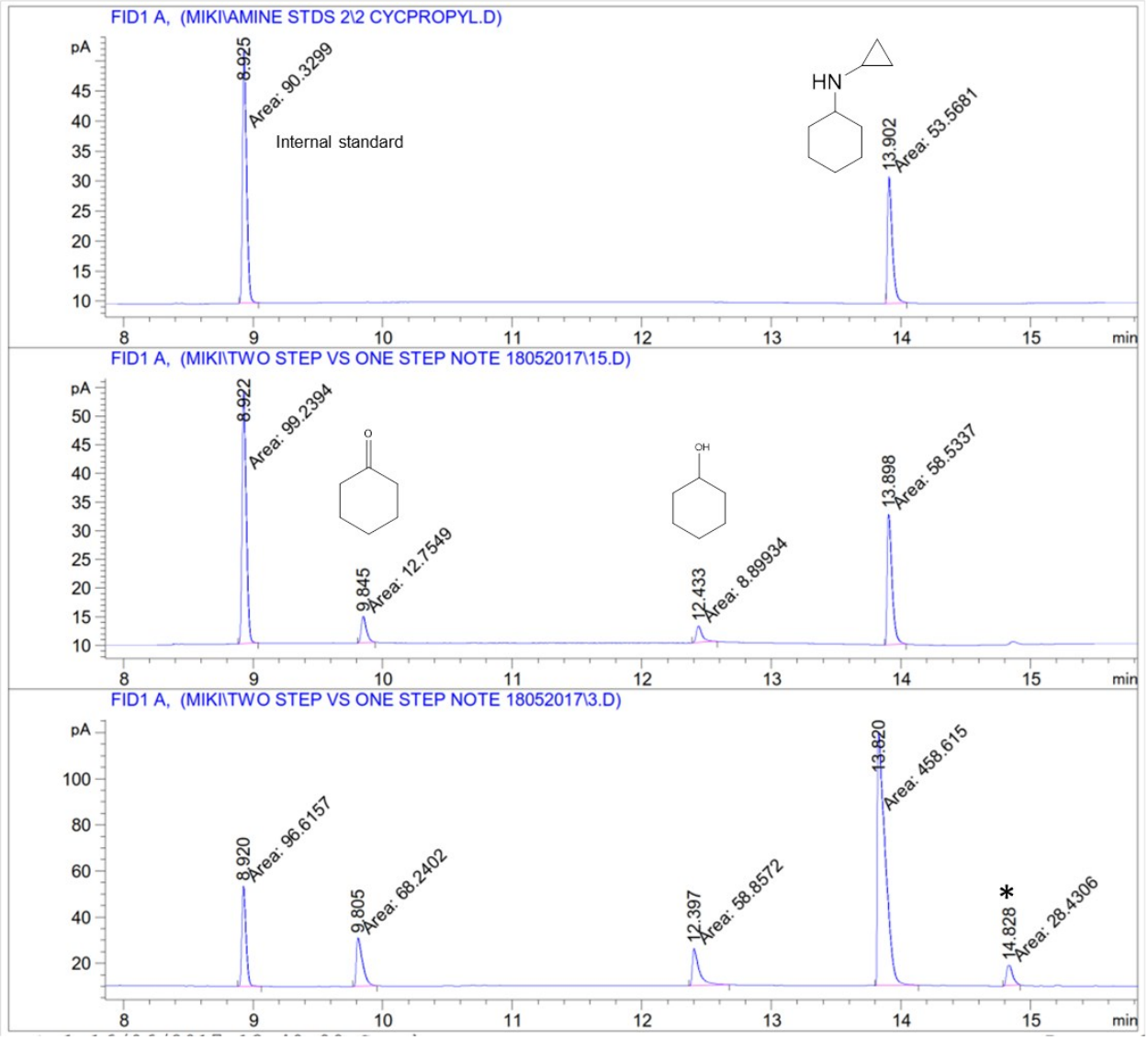
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with propargylamine **c**. Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown.



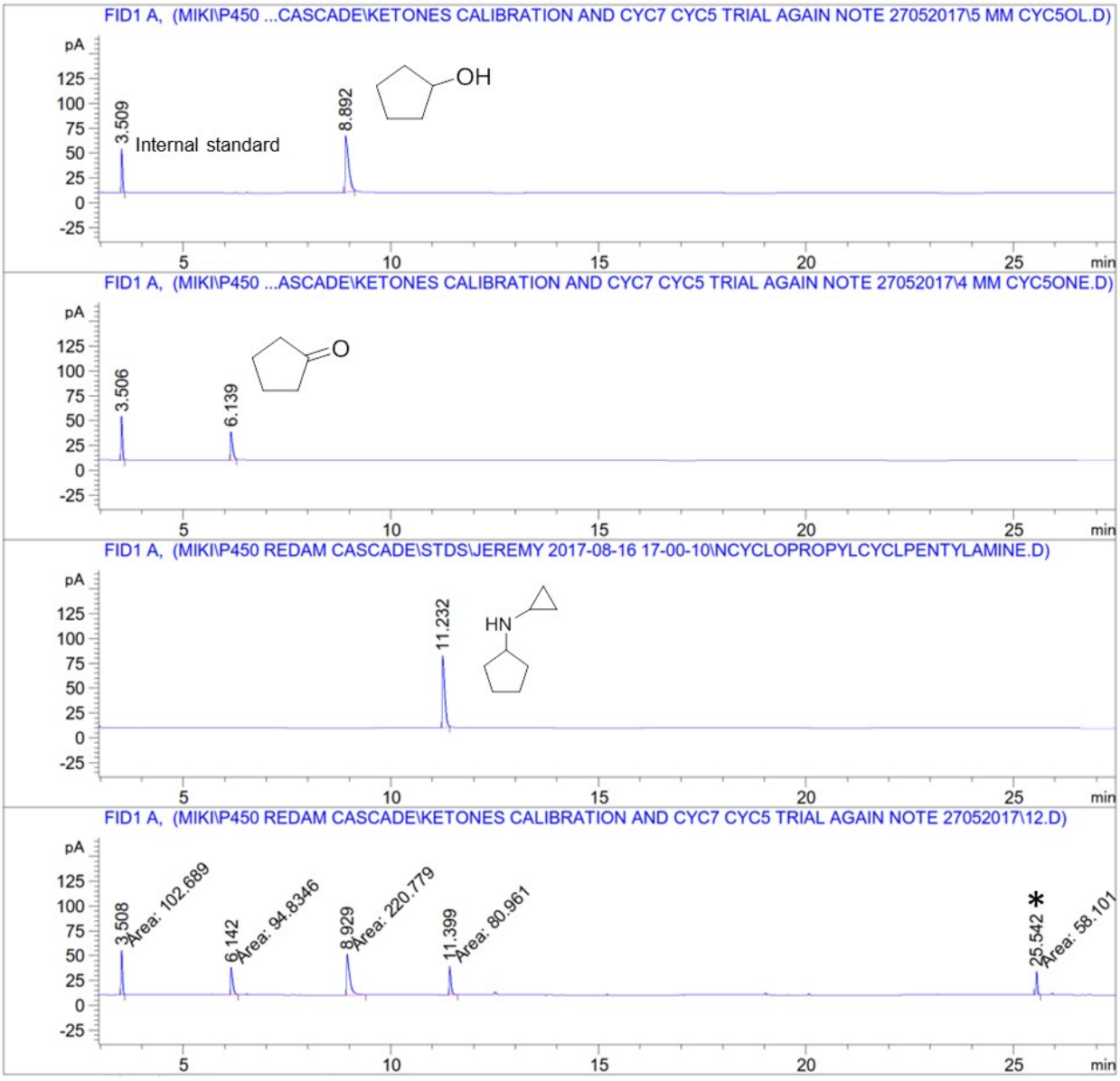
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with propylamine **d**. Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown. An asterisk indicates a putative imine peak. Further details are given in the section “GC-MS traces”.



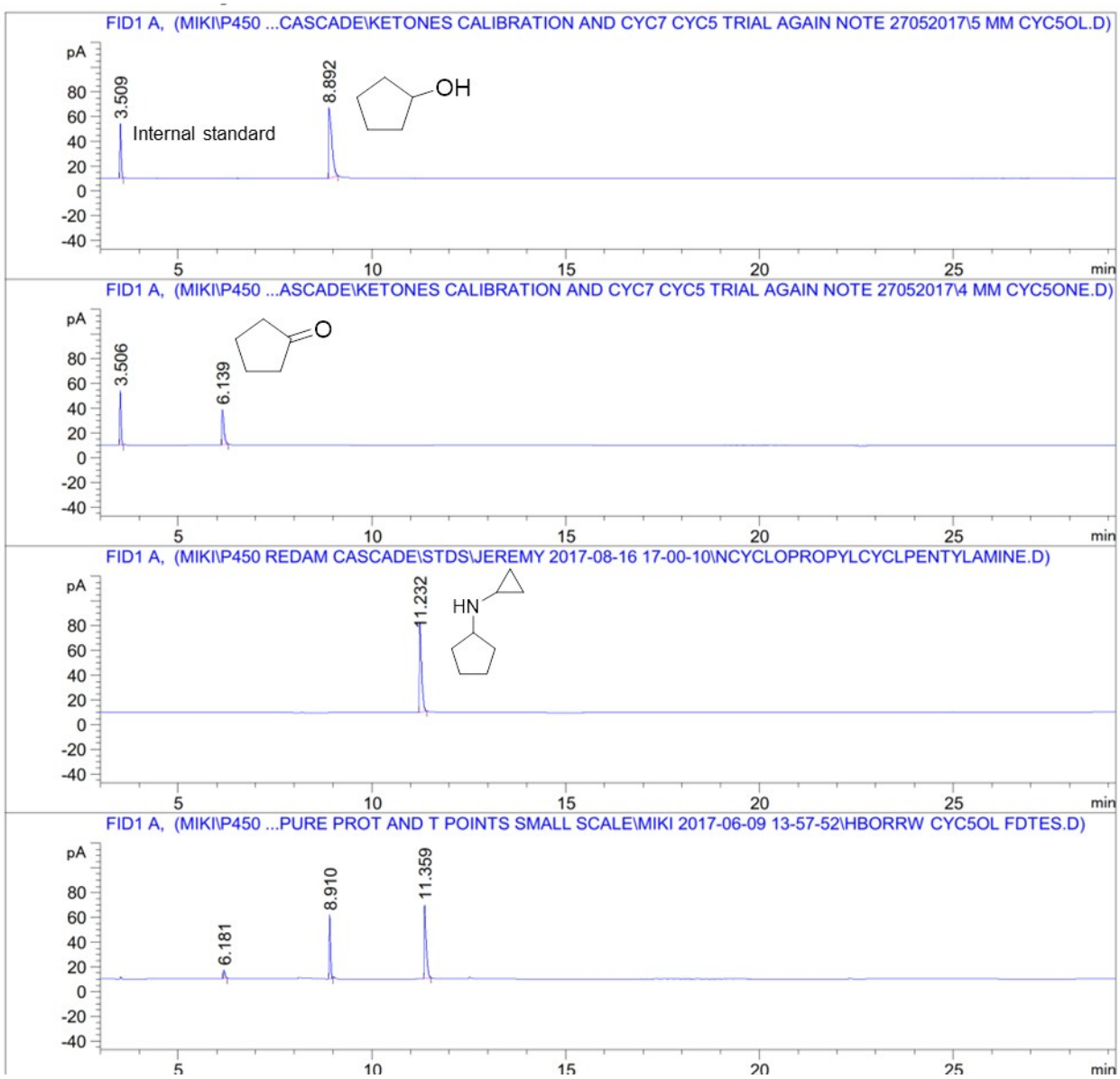
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with allylamine **e**. Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown. An asterisk indicates a putative imine peak.



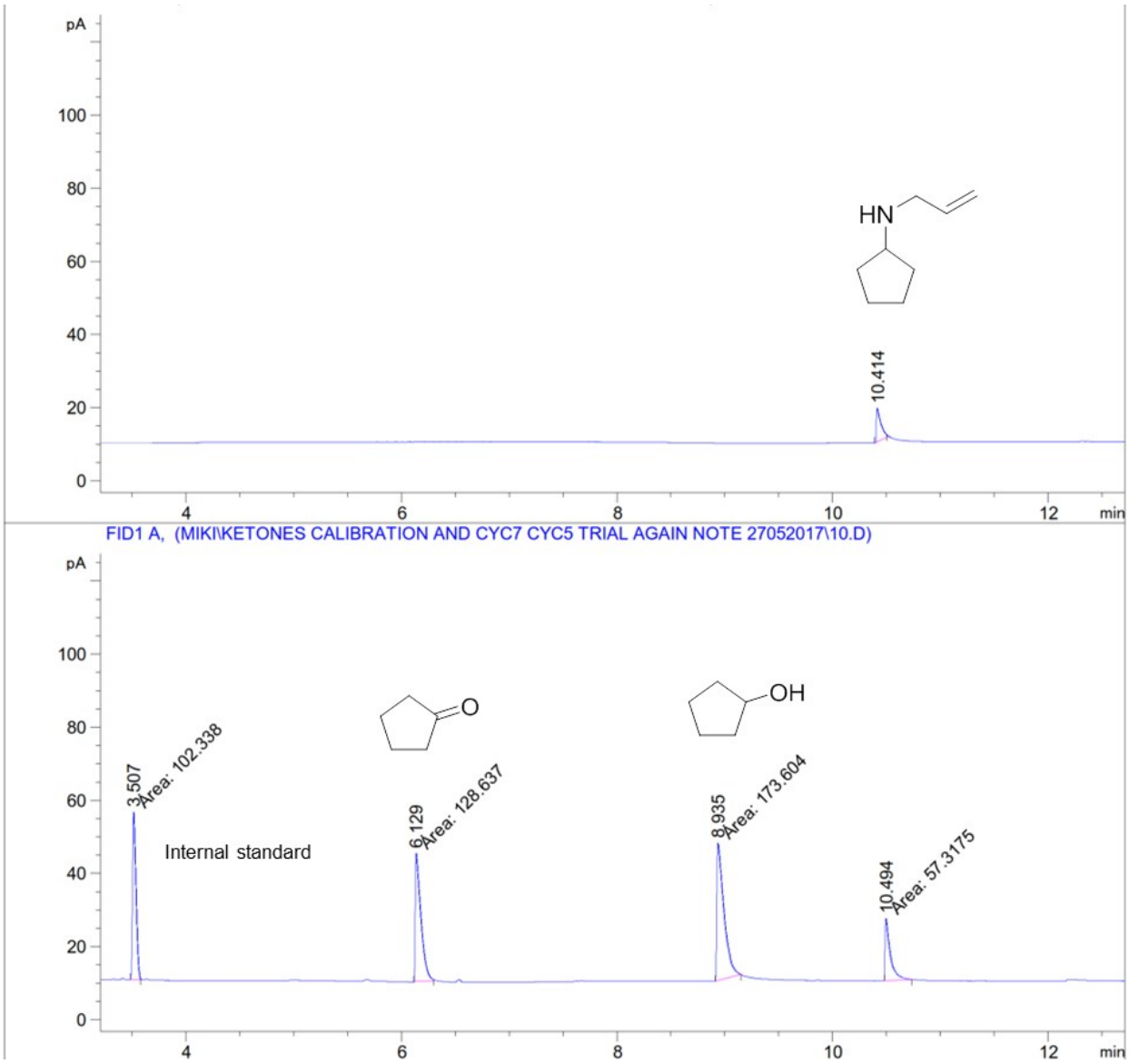
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclohexane **1** with cyclopropylamine **f**. Amine standard (top trace), one-step cascade (middle trace) and two-step cascade (bottom trace) are shown. An asterisk indicates a putative imine peak. Further details are given in the section “GC-MS traces”.



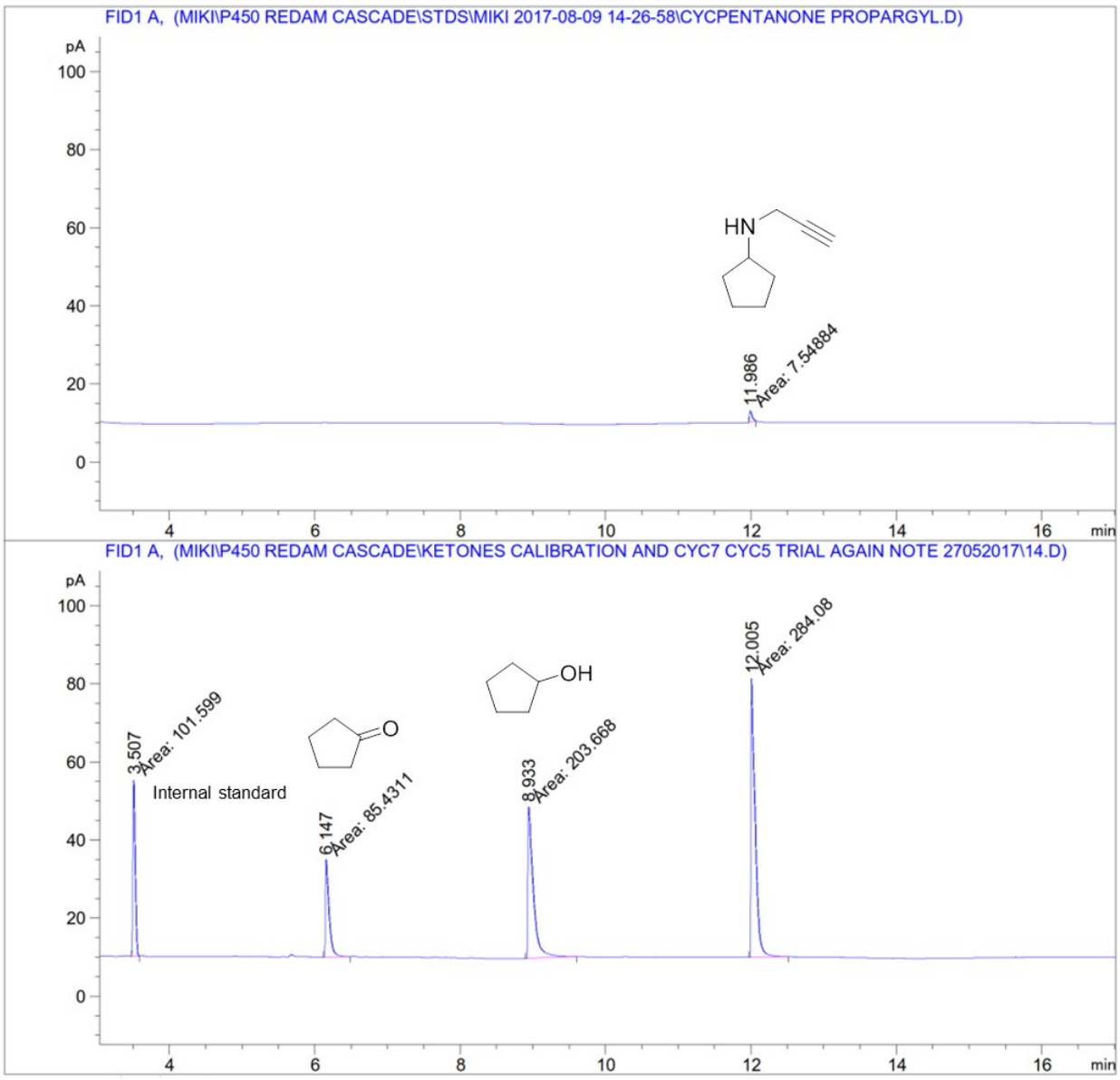
GC-FID chromatogram for the biocatalytic cascade for the amination of cyclopentane **4** with cyclopropylamine **f**. Standards (top, second and third trace) and two-step cascade (fourth trace) are shown. An asterisk indicates an unidentified by-product that is not detected after amination of cyclopentanol *via* hydrogen-borrowing (see GC trace below). Further details are given in the section "GC-MS traces".



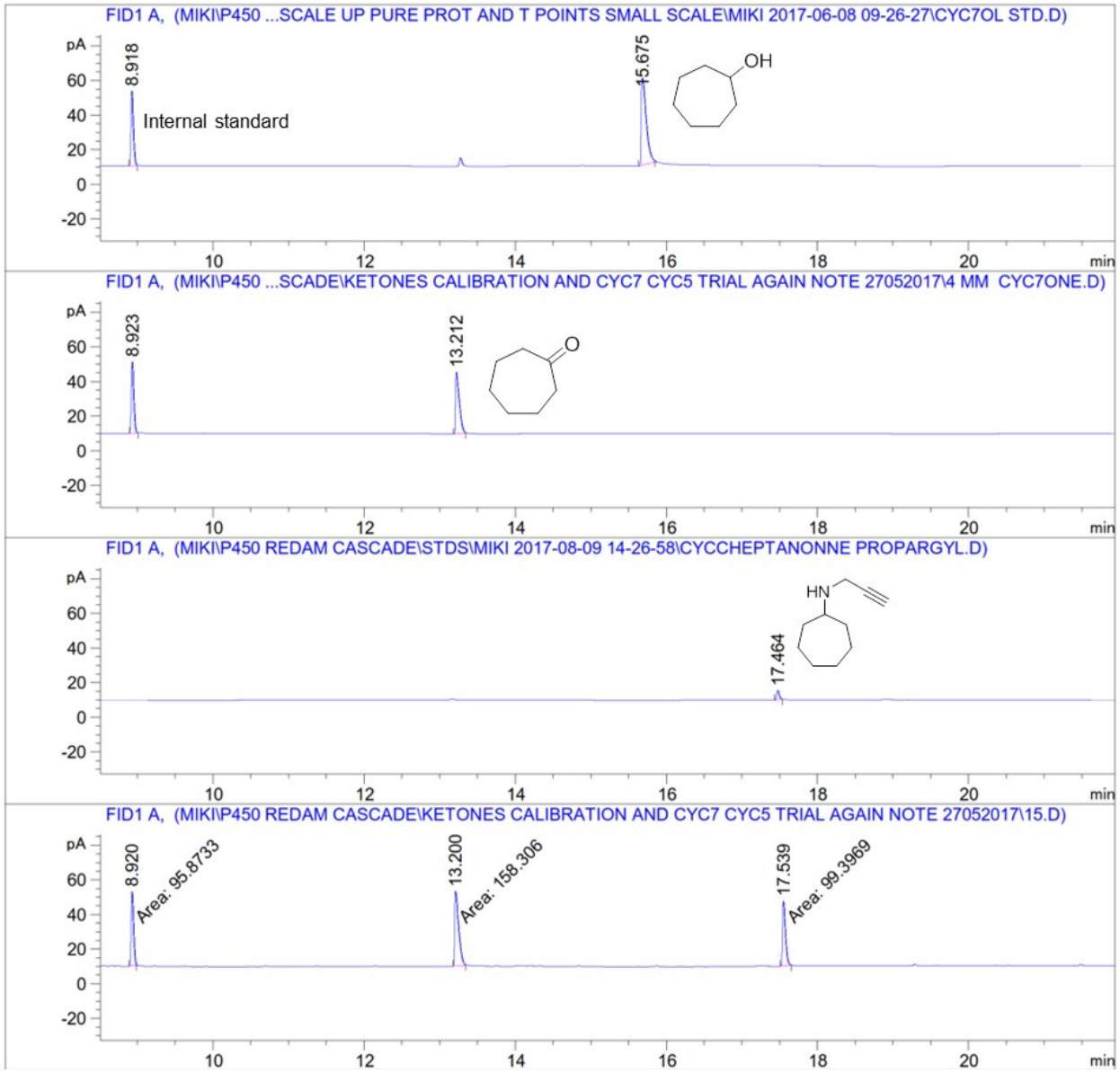
GC-FID chromatogram for *Asp*RedAm/TeSADH W110A-catalysed amination of cyclopentanol **4** with cyclopropylamine **f**, showing standards of (top, second and third trace) and biotransformation (fourth trace).



GC-FID chromatogram for the biocatalytic cascade for the amination of cyclopentane **4** with allylamine **e**. Amine standard (top trace) and two-step cascade (bottom trace) are shown.



GC-FID chromatogram for the biocatalytic cascade for the amination of cyclopentane **4** with propargylamine **c**. Amine standard (top trace) and two-step cascade (bottom trace) are shown.

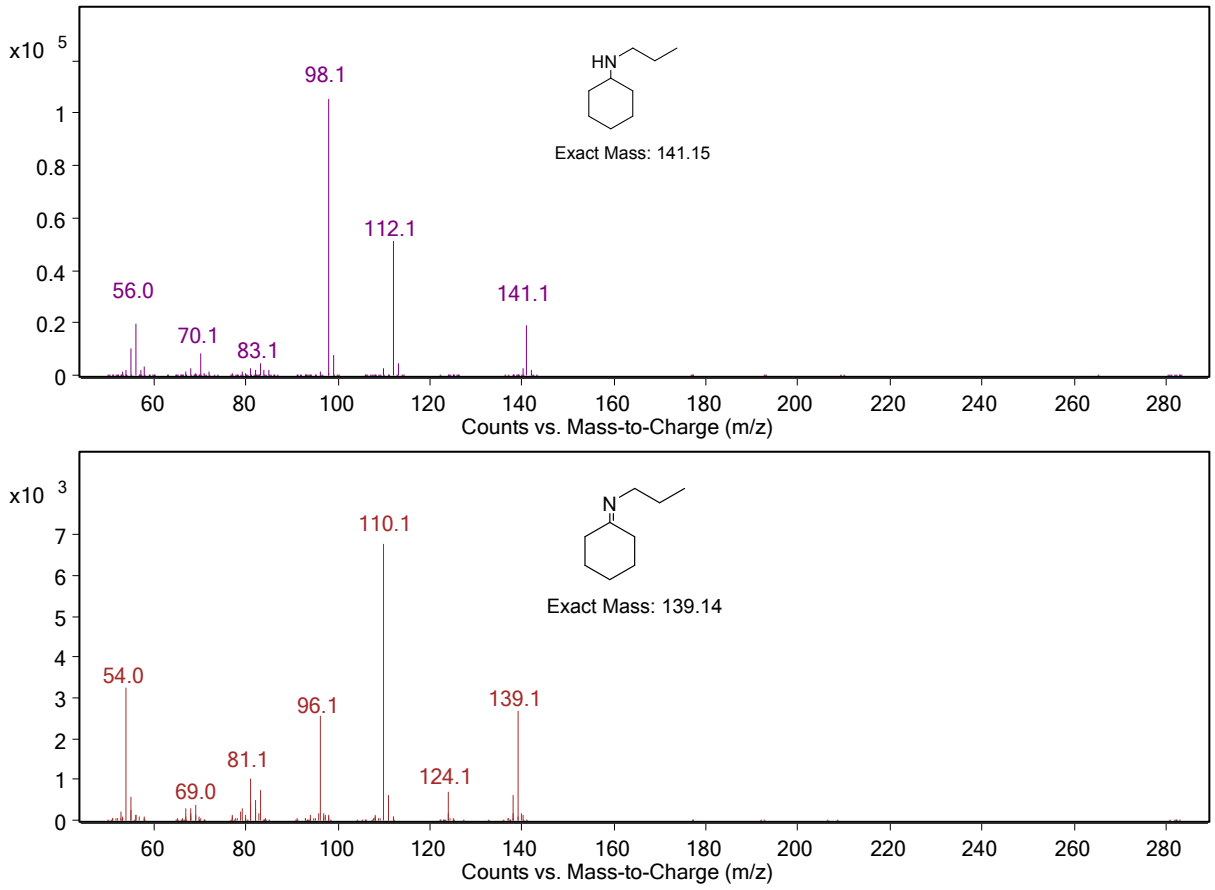


GC-FID chromatogram for the biocatalytic cascade for the amination of cycloheptane **6** with propargylamine **c**. Standards (top, second and third trace) and two-step cascade (fourth trace) are shown.

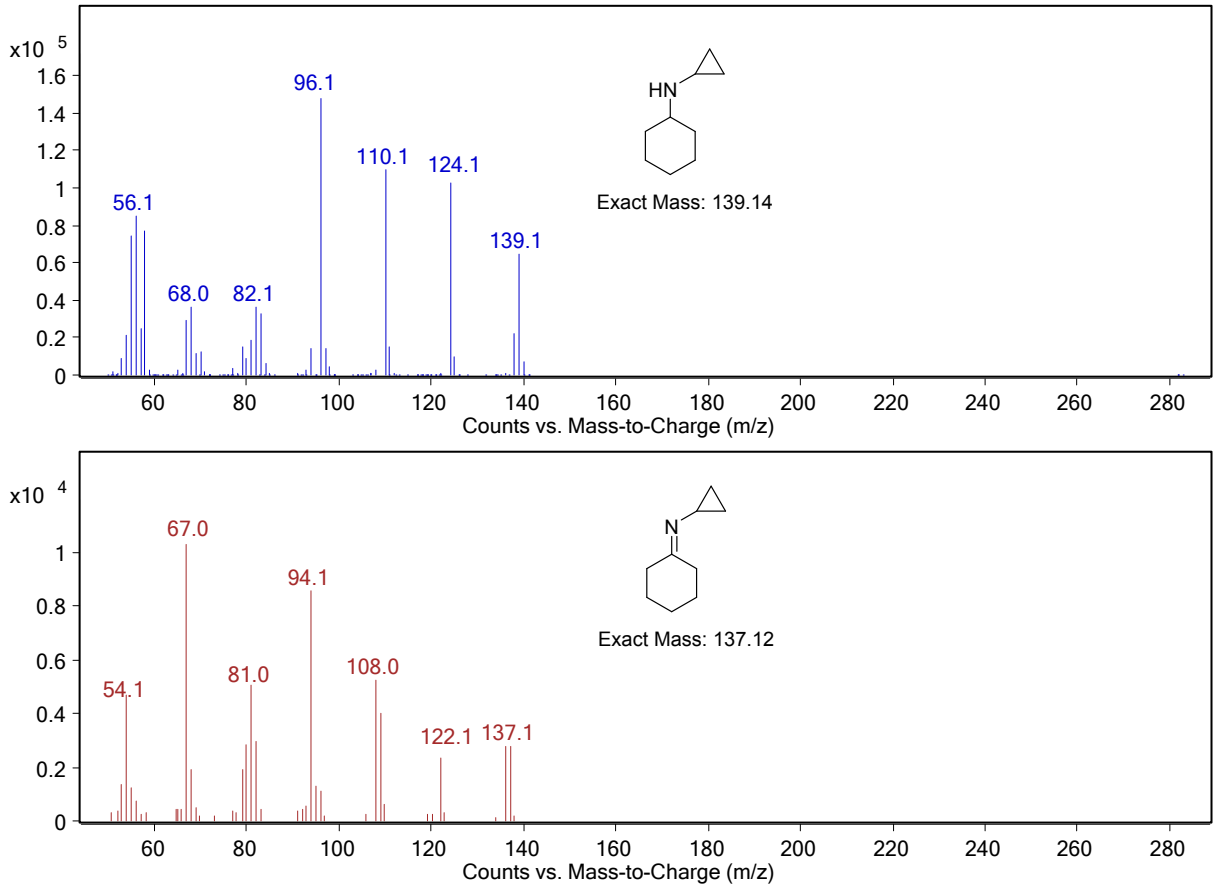
GC-MS traces

By-product analysis

In order to gain a better understanding on the nature of a minor product (indicated with an asterisk in the GC traces above) found in the amination of cyclohexane **1** with amines **d**, **e** and **f**, GC-MS analysis was carried out. Given the fragmentation patterns shown below, we suppose that the additional peak is an imine that is not reduced by the reductive aminase. Unfortunately, it was not possible to detect/resolve the additional peak (approximately 1%) for the reaction carried out with allylamine **e**.

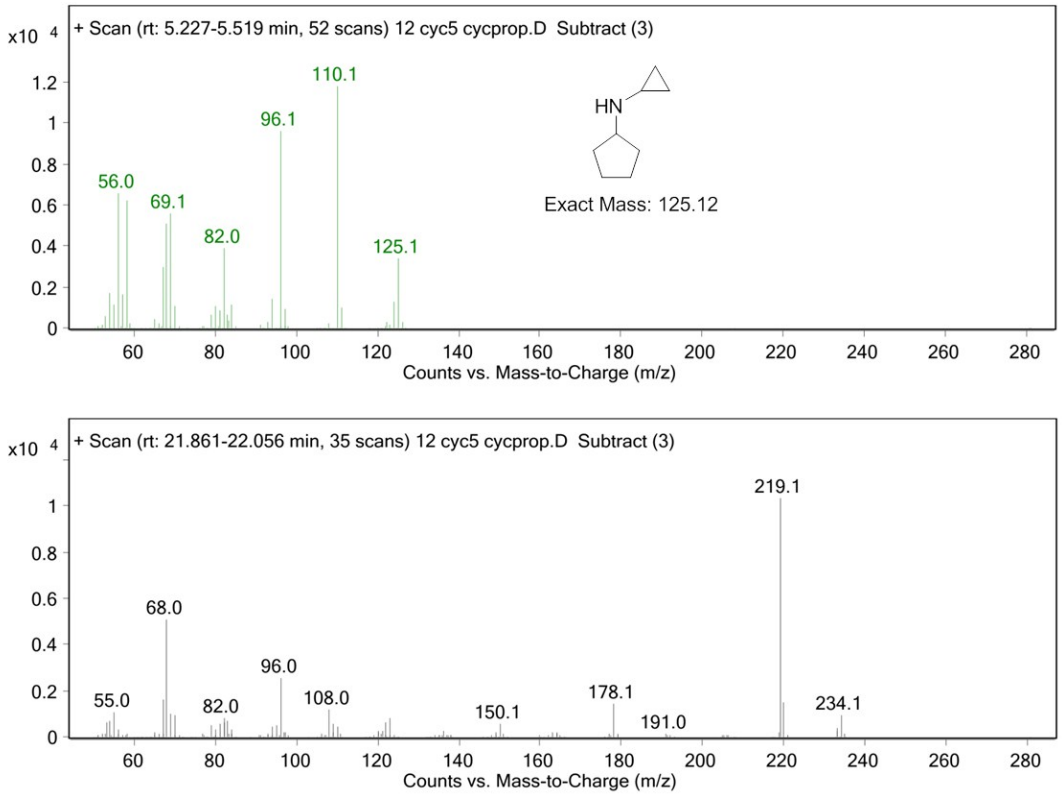


GC-MS analysis: amination of cyclohexane **1** with propylamine **d**. The MS trace for the amine product (top trace) is compared with the MS trace for the additional peak found in GC traces.



GC-MS analysis: amination of cyclohexane **1** with cyclopropylamine **f**. The MS trace for the amine product (top trace) is compared with the MS trace for the additional peak found in GC traces.

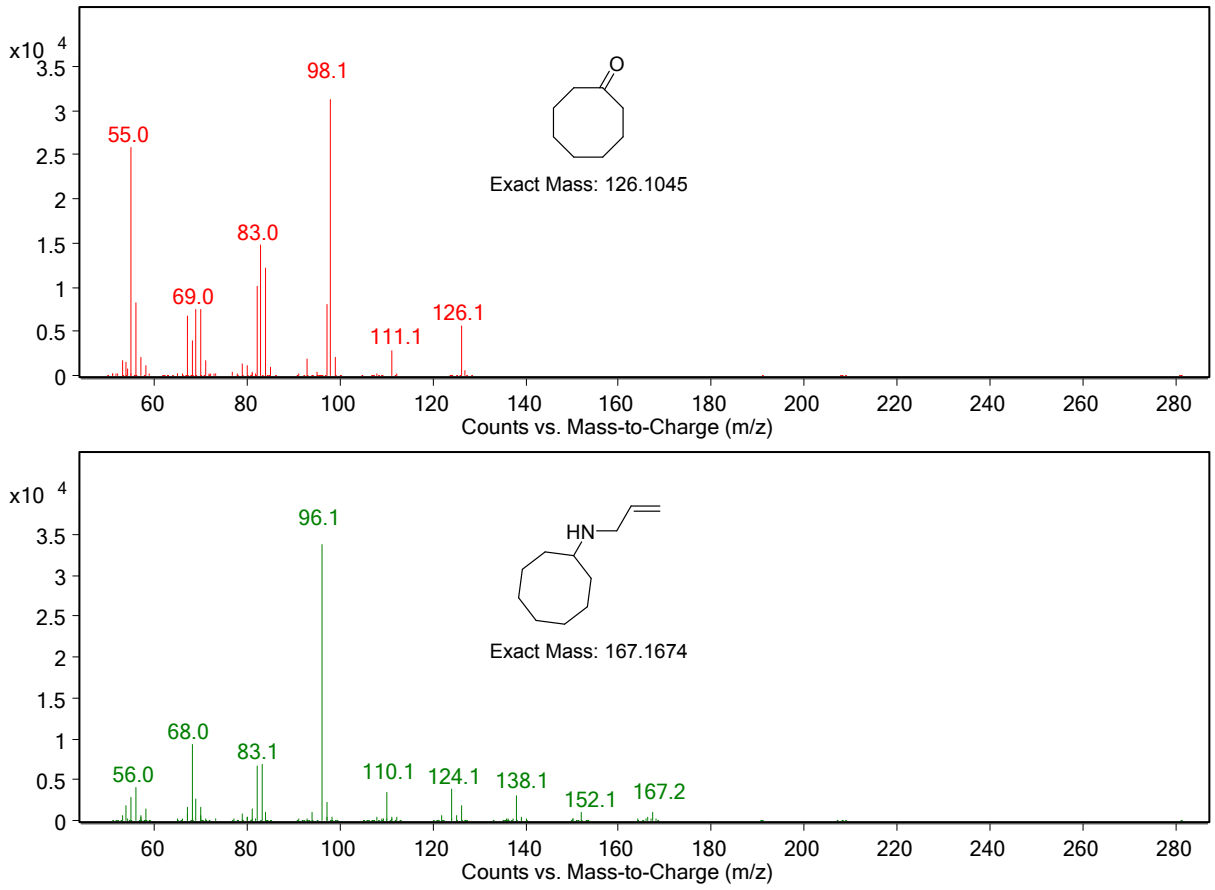
Finally, the amination of cyclopentane **4** with cyclopropylamine **f** was analysed by GC-MS for the identification of the by-product peak observed in biotransformation extracts. The similar fragmentation pattern suggests that the two compounds might be related although further experiments should be done in order to identify this by-product.



GC-MS analysis: Amination of cyclopentane **4** with cyclopropylamine **f**. The MS trace for the amine product (top trace) is compared with the MS trace for the additional peak found in GC traces (bottom trace).

Reductive amination of cyclooctanone with allylamine e

GC-MS analysis suggests that *Asp*RedAm is capable of catalysing reductive amination of cyclooctanone with allylamine **e**.



GC-MS analysis: amination of cyclooctanone with allylamine **e**. The MS trace for the substrate (top trace) is compared with the MS trace for the additional peak found in GC traces.

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2 G. A. Aleku, S. P. France, H. Man, J. Mangas-sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan and N. J. Turner, *Nat. Chem.*, 2017, DOI:10.1038/nchem.2782.

3 A. Pennec, F. Hollmann, M. S. Smit and D. J. Opperman, *ChemCatChem*, 2015, **7**, 236–239.

4 S. L. Montgomery, M. P. Thompson, G. A. Aleku, B. Dominguez and N. J. Turner, *Angew. Chem. Int. Ed.,* 2017, DOI: 10.1002/anie.201705848.