Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Cross-Linking of A Polyketide Synthase Domain Leads to A Recyclable Biocatalyst for Chiral Oxygen Heterocycle Synthesis

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Materials and methods

Chemistry & Analytics. UPLC-MS analysis was performed on an Acquity Ultra Perfomance LC (column: HSS C18, pore size 100 Å, particle size 1.8 µm, 2.1 x 50 mm; PDA detector; SQ detector). Mode of ionisation was ESI+ and found mass is given. Reversed phase-UPLC-applications were performed with membrane-filtrated and double distilled water as well as commercially available UPLC-grade methanol.

Biochemistry. All chemicals and antibiotics were purchased from Sigma-Aldrich or Roth. Cell disruption was conducted by sonication (Sonopuls type HD 3100) from Bandelin.

Chemical synthesis

The synthesis of compounds **1**, **3** and ***rac*-5** was described in reference [1].

Gene expression, enzyme production and conversion experiments

The reported expression plasmid *ambDH3_pET28a(+)* was used for expression of *ambDH3* in *E. coli* BL21(DE3) under analogous conditions as in [2]. 1 g of *E. coli* BL21(DE3) cells carrying *ambDH3_pET28a(+)* were suspended in 10 mL sodium phosphate buffer (100 mM sodium phosphate, pH 7.4) or HEPES buffer (25 mM HEPES, 100 mM NaCl, pH 6.8). Cell disruption was performed on ice by sonication (45% amplitude, 10 cycles, 30 s sonication, 30 s pause). After centrifugation (10000 g, 4 °C, 30 min), the obtained crude lysate was filtered (cellulose acetate, 0.45 µm) and either used for enzymatic conversions or formation of cross-linked enzyme aggregates. Calibration straights of **1** and **2** were used to correlate the areas in the UPLC-MS chromatograms to their molar ratio in all experiments. All small-scale conversions were performed in a 2 mL tube containing substrate **1** (0.1 mg, concentration 2 mM) and free or immobilised enzyme in sodium phosphate or HEPES buffer. For enzyme reactions with soluble AmbDH3, the protein containing lysate was carefully inverted and 40 µL added to the substrate.

Preparation of AmbDH3-CLEA

The preparation of AmbDH3-CLEA was performed slightly modified according to Sewald *et al.*.[3] Ammonium sulphate (472 g per liter) was added to the cell lysate. The solution was incubated under mild agitation at 4 °C in an overhead rotor at 15 rpm. After 1 h, a 25% (v/v) glutaraldehyde solution was added to a final concentration of 0.5% and agitation continued for 2 h at 4 °C. The AmbDH3-CLEA solution was centrifuged (10000 g, 4 °C, 30 min) and the supernatant discarded. The residual CLEA pellet was washed three times with sodium phosphate buffer (200 µL or 5 mL) and centrifuged (10000 g, 4 °C, 15 min). After discarding the supernatant, the pellet was stored at 4 °C until usage. The subsequent enzymatic reactions were carried out with the equivalent amount of catalyst resulting from 0.4 g of pellet (4 mL cell-free lysate or 5 mL CLEA solution) per 10 mg of **1**.[2] For conversions on the analytical or semi-preparative scale, the corresponding amount of AmbDH3-CLEA solution (4 mg or 0.4 g of initially used cell pellet from the induced expression culture, equivalent to 50 µL or 5 mL CLEA solution) was aliquoted in 2 or 15 mL tubes prior the following washing steps.

For the optimisation of preparation conditions different buffers and glutaraldehyde concentrations were used for cross-linking and followed enzyme reactions. Sodium phosphate buffer (100 mM sodium phosphate, pH 7.4) and HEPES buffer (25 mM HEPES, 100 mM NaCl, pH 6.8) were tested, as well as different final glutaraldehyde concentrations during cross-linking (0.5, 1, 2 and 5%). The CLEAs were obtained in form of fine, faint orange powders in all cases (Figure S1).

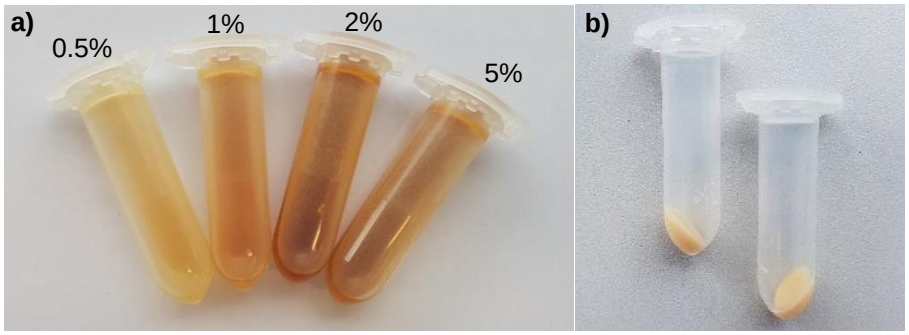


Figure S1. a) Preparation of AMbDH3 using different GA concentrations. b) AmbDH3 after washing.

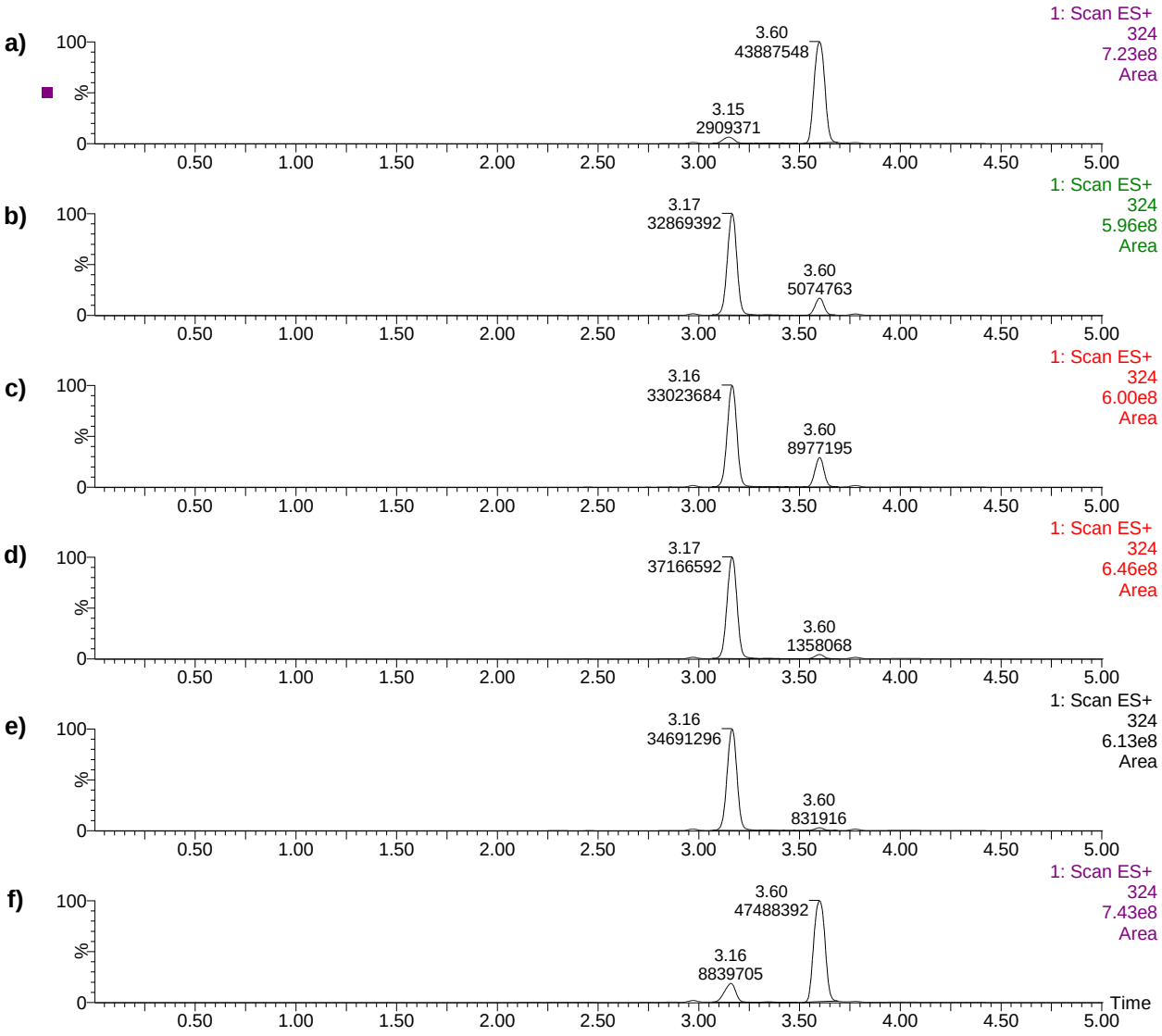


Figure S2. UPLC-MS analysis of conversion experiments of the individual fractions from AmbDH3-CLEA preparation with **1**. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.15–3.17 min, t_R (**2**) = 3.60 min. **a)** Lysate (90%), **b)** after ammonium sulphate precipitation (10%), **c)** washing step 1 (16%), **d)** washing step 2 (3%), **e)** washing step 3 (2%) and **f)** AmbDH3-CLEA (88%). Conversion values are given in brackets.

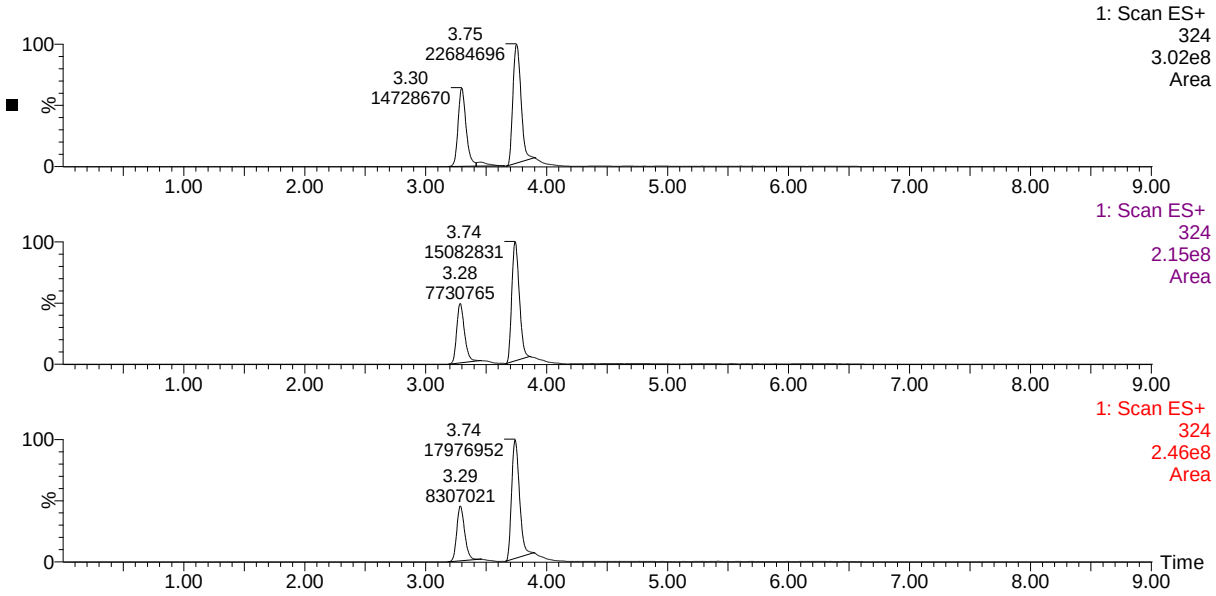


Figure S3. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA (cross-linked with 0.5% glutaraldehyde) in HEPES buffer after 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.28–3.30 min, t_R (**2**) = 3.74–3.75 min.

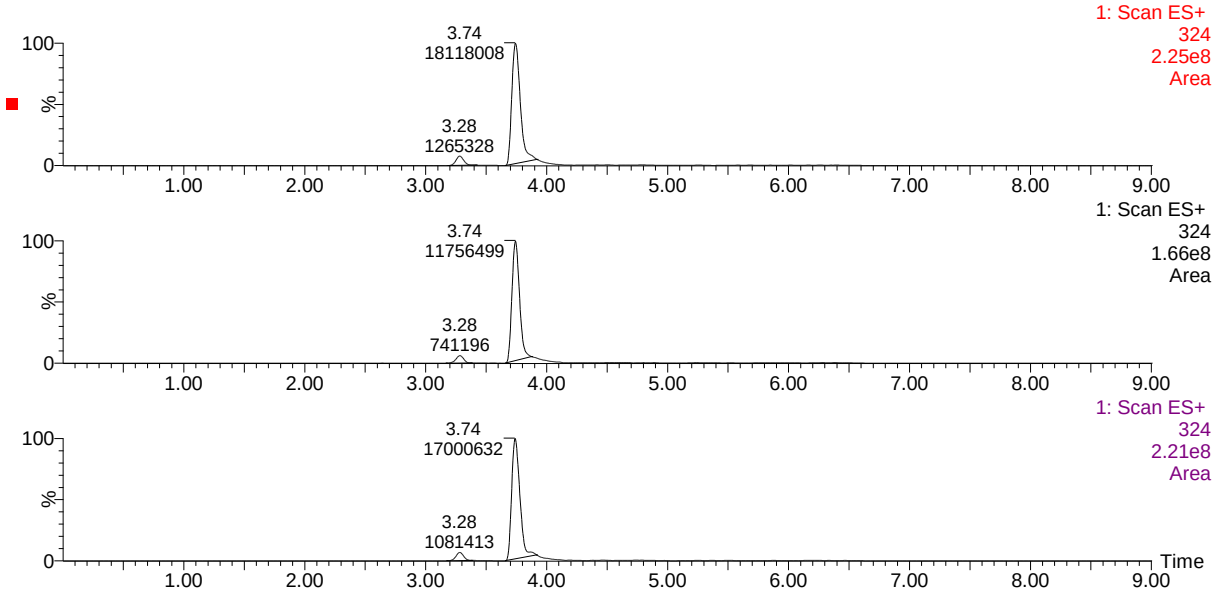


Figure S4. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA (cross-linked with 0.5% glutaraldehyde) in sodium phosphate buffer after 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.28 min, t_R (**2**) = 3.74 min.

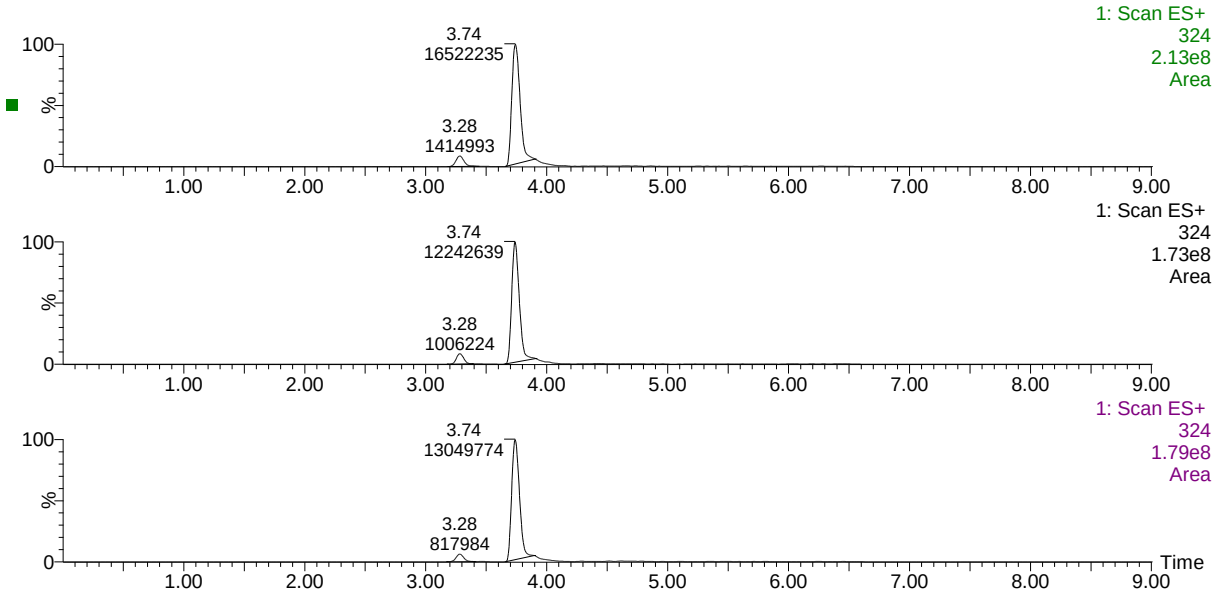


Figure S5. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA (cross-linked with 1% glutaraldehyde) in sodium phosphate buffer after 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.28 min, t_R (**2**) = 3.74 min.

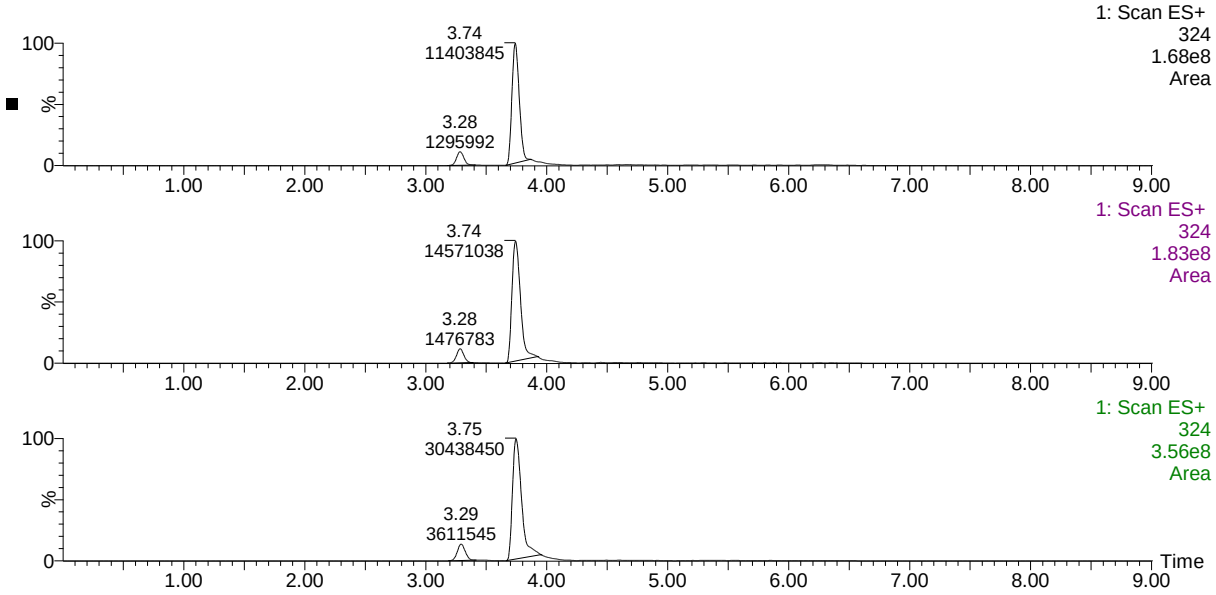


Figure S6. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA (cross-linked with 2% glutaraldehyde) in sodium phosphate buffer after 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.28–3.29 min, t_R (**2**) = 3.74–3.75 min.

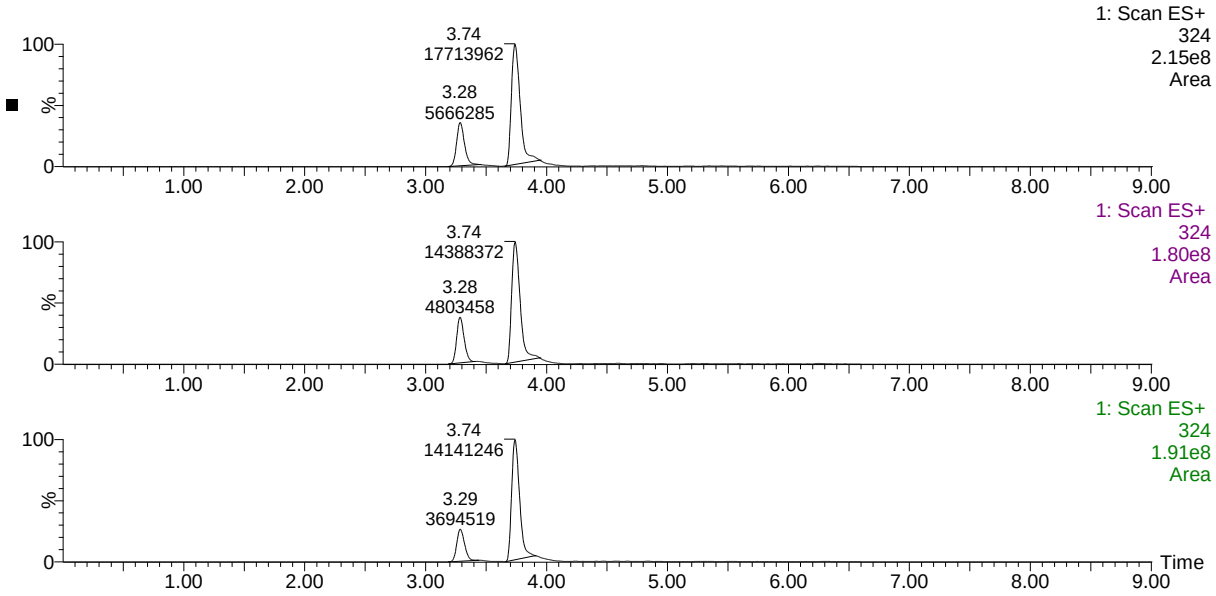
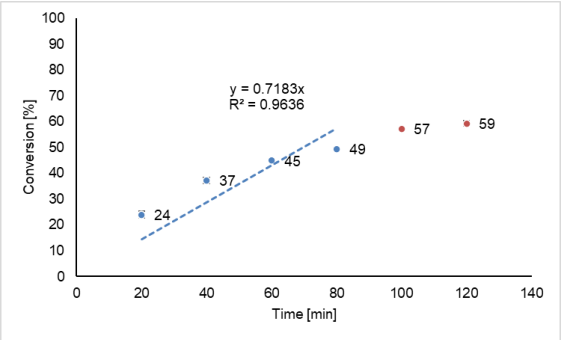


Figure S7. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA (cross-linked with 5% glutaraldehyde) in sodium phosphate buffer after 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.28–3.29 min, t_R (**2**) = 3.74 min.

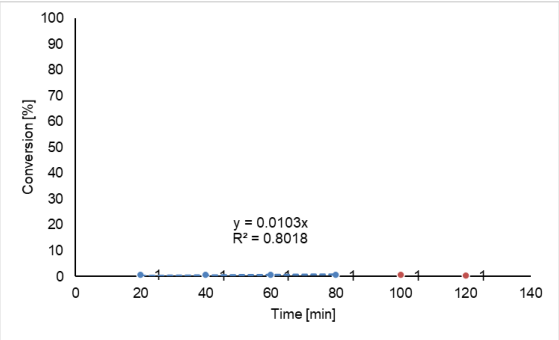
Determination of activity recovery

The reactions of the lysate, the precipitation and washing fractions and the CLEA were conducted in 2 mL tubes as triplicates in a final volume of 166 µL with 0.1 mg substrate **1** (final concentration 2 mM). 40 µL of the cleared lysate or 50 µL of the precipitation and washing fraction (due to increase of volume) were incubated with **1** for up to 2 h at 30 °C and 300 rpm. For the reaction of AmbDH3-CLEA the appropriate amount of CLEA was resuspended in sodium phosphate buffer and transferred to the tube with the substrate. The reactions were quenched in 20 min intervals by addition of EtOAc (500 µL). The stopped solutions were immediately extracted and the solvent evaporated. The remaining solid was solved in MeOH and analysed *via* UPLC-MS. The conversion was calculated based on the integrals of **1** and **2** using a calibration curve.

Lysate:



Precipitation supernatant:



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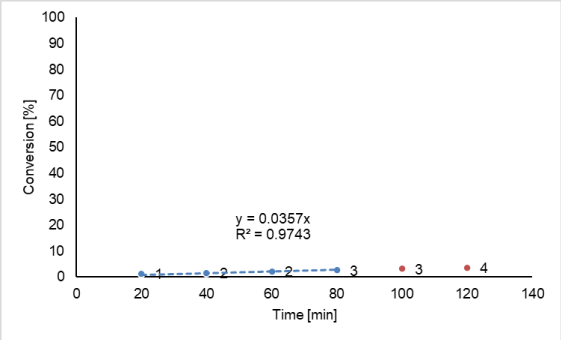


AmbDH3-CLEA:

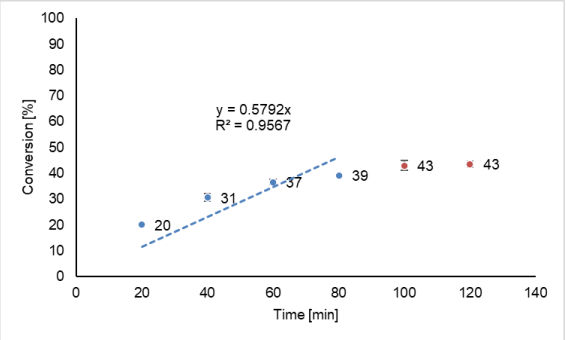


Figure S8. Conversion of **1** into **2** by the individual fractions of AmbDH3-CLEA preparation over the time. Only the first four values at 20, 40, 60 and 80 min were in the linear range and thus used for the activity calculation.

Table S1. Values for the slopes of the linear equations of the conversion with substrate **1** for up to 80 min incubation time with different fractions during the preparation of AmbDH3-CLEA.

Slope of linear equation			
Lysate	Precipitation	Washing	CLEA
0.7183	0.0103	0.0357	0.5792

For determination of the immobilisation yield, activity recovery and immobilisation efficiency, the following equations were used.[4] A_{lysate} corresponds to the activity of the cleared lysate, $A_{precipication}$ is the activity of the supernatant after precipitation with ammonium sulphate, $A_{washing}$ corresponds to the activity of the supernatant after washing the CLEAs with sodium phosphate buffer and A_{CLEA} is the activity of the resulting AmbDH3-CLEA.

Immobilisation yield [%] = $100 \times \frac{\text{immobilised activity}}{\text{starting activity}} = 100 \times \frac{A_{lysate} - A_{precipitation} - A_{washing}}{A_{lysate}}$

Immobilisation efficiency [%] = $100 \times \frac{\text{observed activity}}{\text{immobilised activity}} = 100 \times \frac{A_{CLEA}}{A_{lysate} - A_{precipitation} - A_{washing}}$

Activity recovery [%] = $100 \times \frac{\text{observed activity}}{\text{starting activity}} = 100 \times \frac{A_{CLEA}}{A_{lysate}}$

Table S2. Determination of the activity recovery, immobilisation yield and immobilisation efficiency.

A_{lysate}	A_{CLEA}	$A_{precipitation}$	$A_{washing}$
2.4×10^{-3} u	1.9×10^{-3} u	34×10^{-6} u	119×10^{-6} u

Activity Recovery [%]	Immobilisation yield [%]	Immobilisation efficiency [%]
81	94	86

Calculation of AmbDH3-loading

1 g of expression cells were suspended in 10 mL HEPES buffer and cell disruption was performed on ice by sonication (45% amplitude, 10 cycles, 30 s sonication, 30 s pause). After centrifugation (10000 g, 4 °C, 30 min), the obtained crude lysate was filtered (cellulose acetate, 0.45 µm), passed through a HisTrap™ FF Ni NTA column (in combination with an FPLC Äkta Pure System) and washed with wash buffer I (30 mm Tris, 500 mm NaCl, 10% glycerol, pH 7.5) or II (30 mm Tris, 500 mm NaCl, 10 mm imidazole, 10% glycerol, pH 7.5). Elution of the target protein was achieved using a linear gradient from 0 to 100% elution buffer (30 mm Tris, 500 mm NaCl, 500 mm imidazole, 10% glycerol, pH 7.5). All protein-containing fractions were combined to determine the amount of overall protein. The preparation of AmbDH3-CLEAs was carried out as described above and the amount of overall protein in all individual fractions was determined. The protein concentration was determined by Bradford protein assay in all cases and the protein amount calculated based on the volume of the samples.

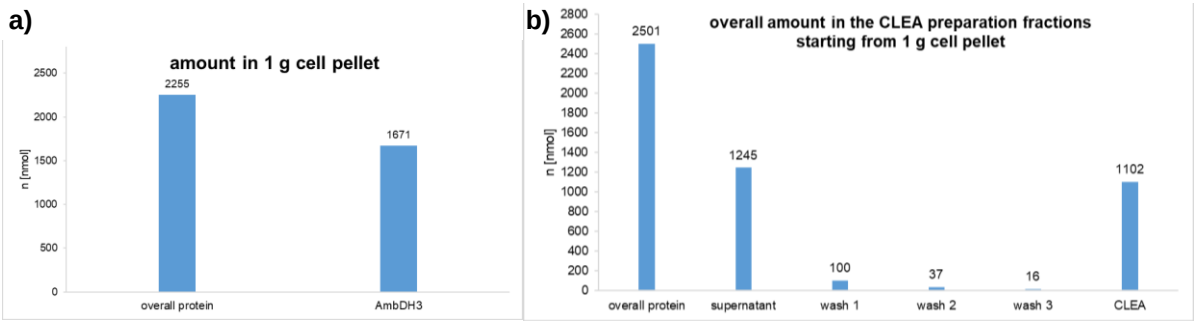


Figure S9. a) Amount of AmbDH3 in the overall protein from 1 g induced cells of an *ambDH3* expression culture. b) Amount of overall protein in the individual fractions of the CLEA preparation. All values were determined by Bradford protein assay.

The absolute amount of AmbDH3 in 1 g of cells was 1.67 µmol giving a proportion of 74% in the overall isolable protein of an induced *ambDH3* expression culture (Figure S9a). Based on this, a homogenous standard reaction with 332 nmol (0.1 mg) of **1** was calculated to contain 6.68 nmol (230 µg, M (AmbDH3) = 34.397 kDa) AmbDH3.

The overall protein in the CLEA obtained from 1 g of induced *ambDH3* expression culture was calculated to be 1.10 µmol (44%) by subtracting the values of the supernatant as well as the three washing fractions from the overall amount in the lysate applied (Figure S9b). Based on the ratio of AmbDH3 in the overall protein determined above and based on the assumption that AmbDH3 would rather accumulate in the solid fraction during ammonium sulphate precipitation and cross-linking, the amount of AmbDH3 in the CLEA was 0.82–1.10 µmol, which corresponds to 44–60% of the original amount in the lysate. A heterogenous standard reaction with 332 nmol (0.1 mg) of **1** thus contains 3.27–4.42 nmol (113–152 µg) AmbDH3.

Kinetic analysis

Steady state kinetic data were determined as described by Sherman *et al*.[5] and Hahn *et al.*[1] The appropriate amount of **1** was dissolved in DMSO (2 µL) and diluted with sodium phosphate buffer (48 µL, pH 7.4, 100 mM sodium phosphate) for 10 min at 37 °C and 300 rpm. The substrate solution was added to aliquots of AmbDH3-CLEA (0.11 mg, 3.27 nmol) and led to a final protein concentration of 65.4 µM and a final substrate concentration of 0.07–1.19 mM (the maximum substrate concentration was limited by the substrate solubility). The mixture was incubated for 8 min at 37 °C and 300 rpm. 10 µL of the mixture were diluted with 990 µL H_2O/MeCN (1:1), filtered and analysed by UPLC-MS. Samples with a substrate concentration above 1 mM were further diluted (100 µL mixture + 900 µL H_2O/MeCN 1:1) before being analysed. All experiments were conducted in triplicate.

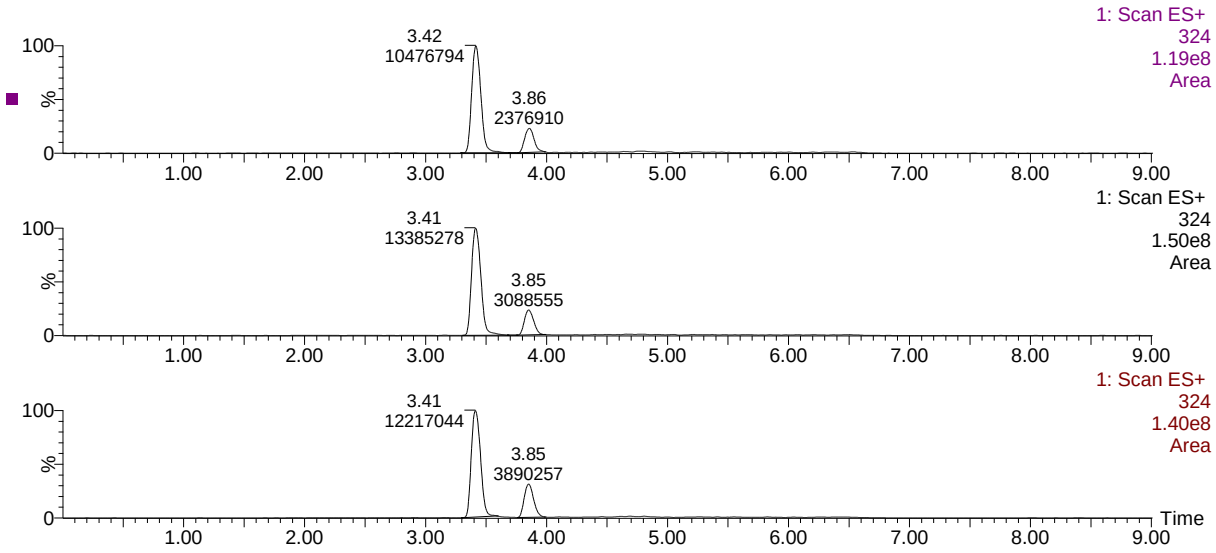


Figure S10. UPLC-MS analysis of the conversion of **1** (0.07 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.84–3.85 min.

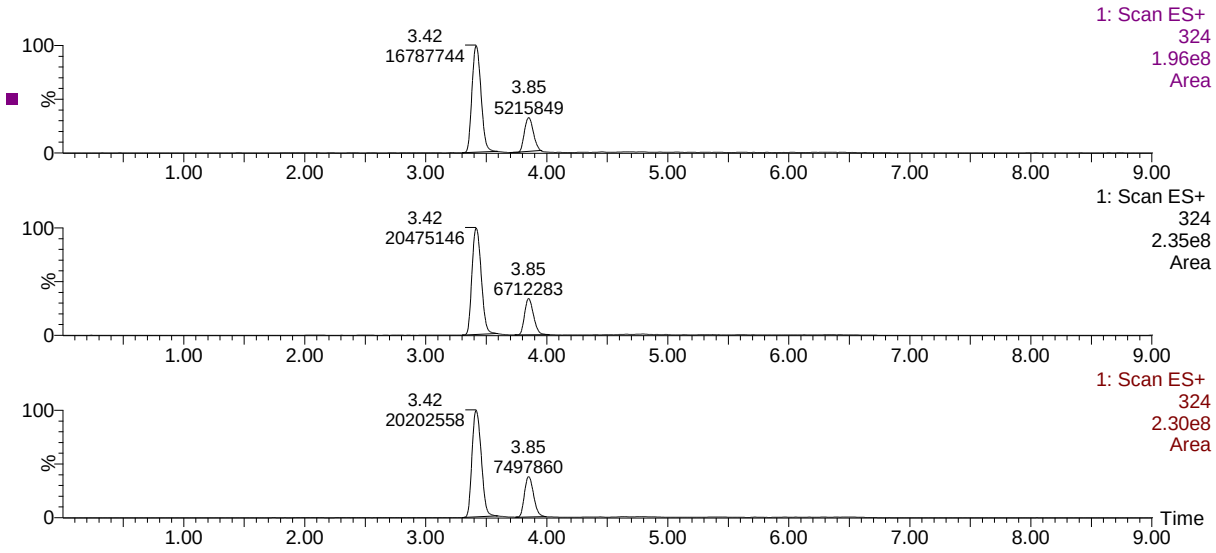


Figure S11. UPLC-MS analysis of the conversion of **1** (0.15 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.42 min, t_R (**2**) = 3.85 min.

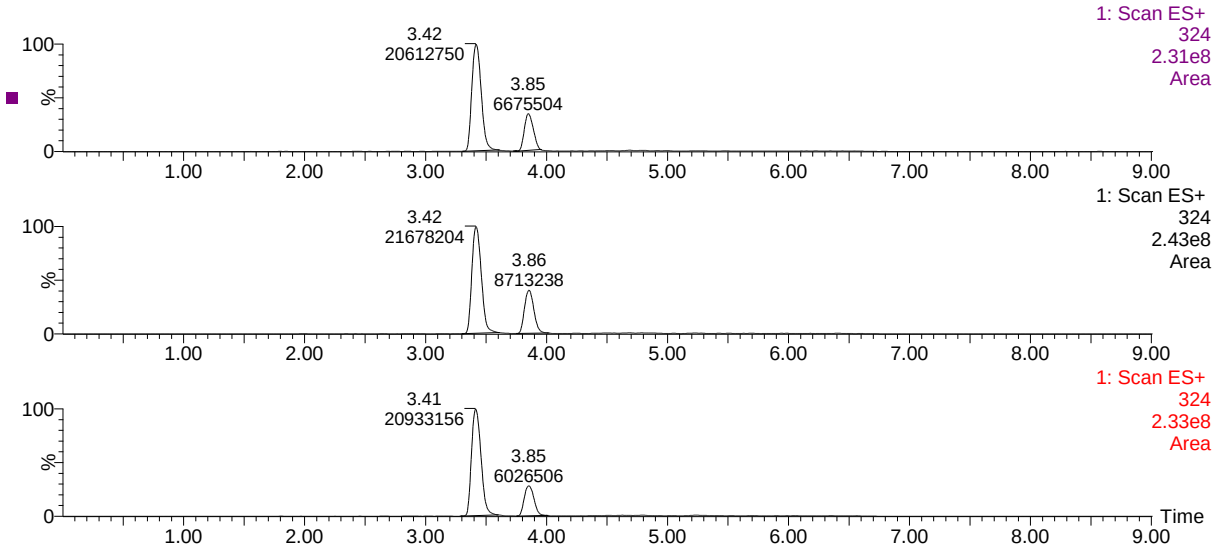


Figure S12. UPLC-MS analysis of the conversion of **1** (0.23 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.85–3.86 min.

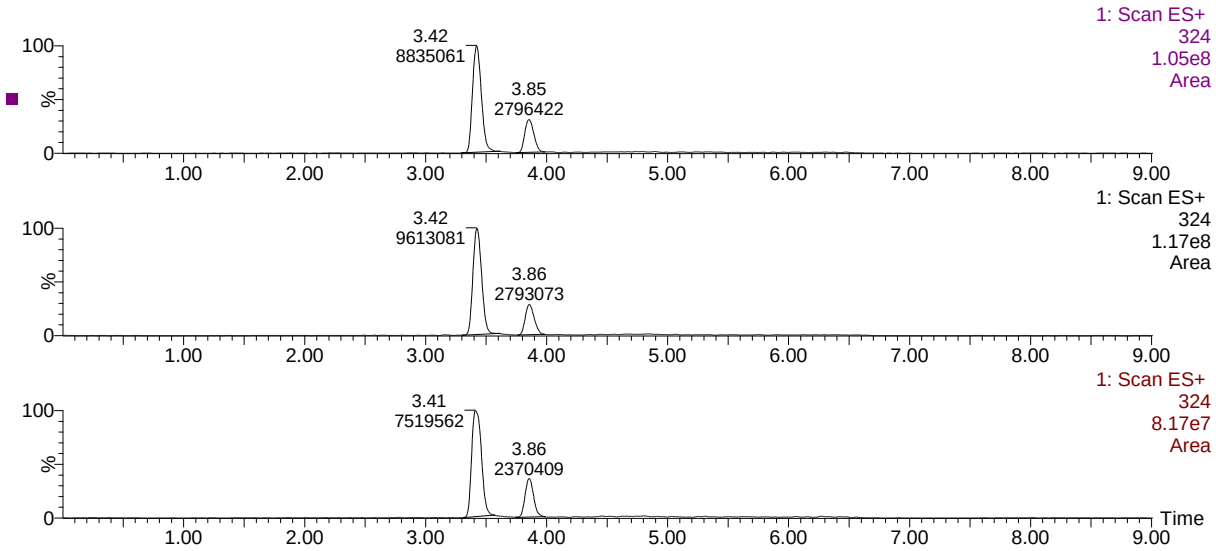


Figure S13. UPLC-MS analysis of the conversion of **1** (0.52 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.85–3.86 min.

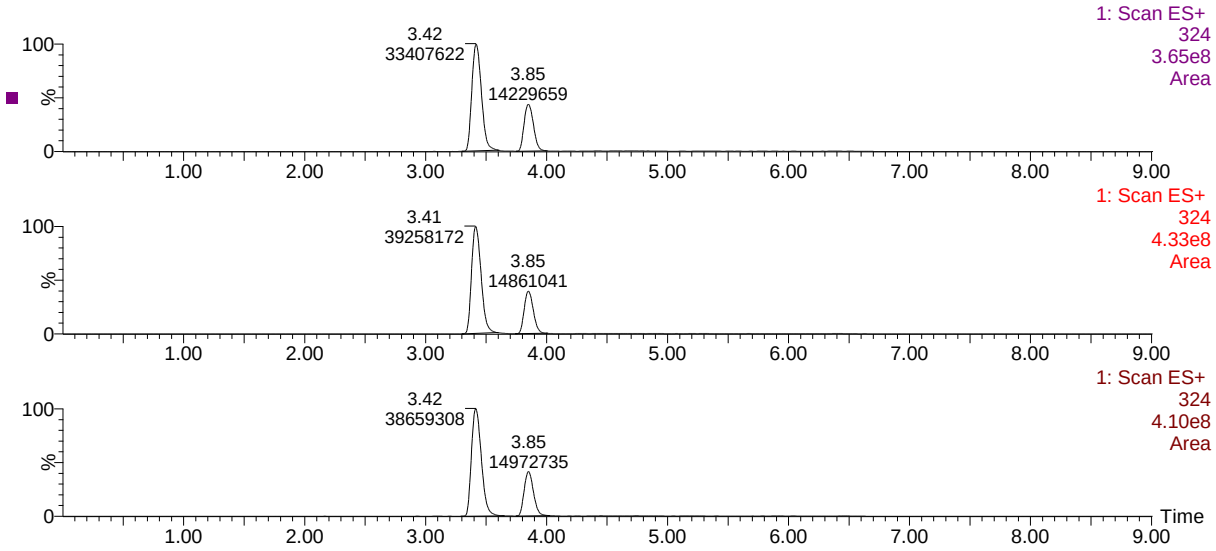


Figure S14. UPLC-MS analysis of the conversion of **1** (0.62 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.85 min.

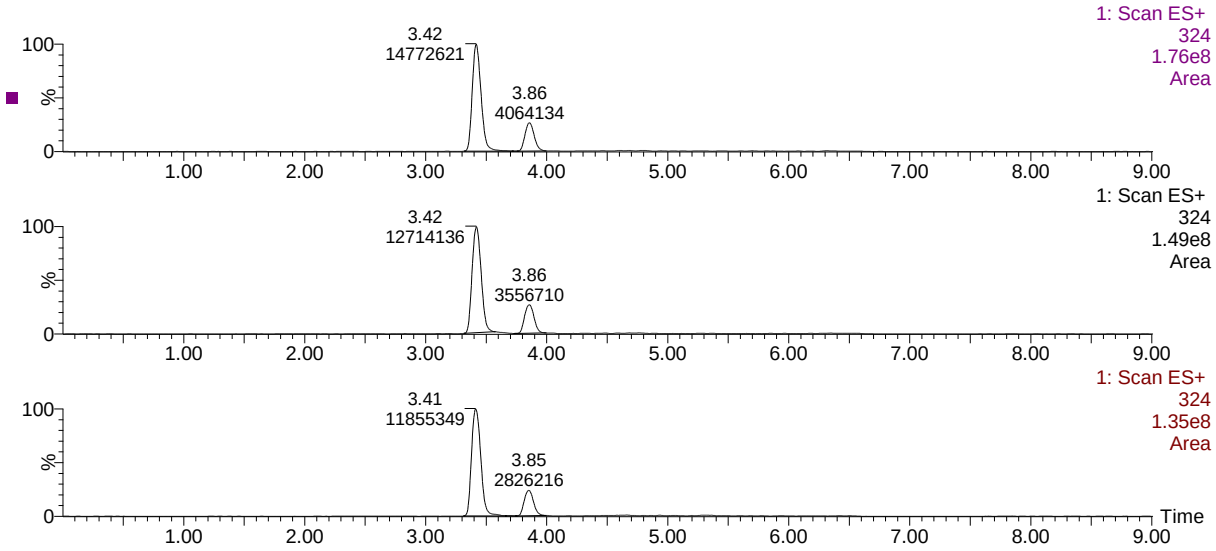


Figure S15. UPLC-MS analysis of the conversion of **1** (0.79 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.85–3.86 min.

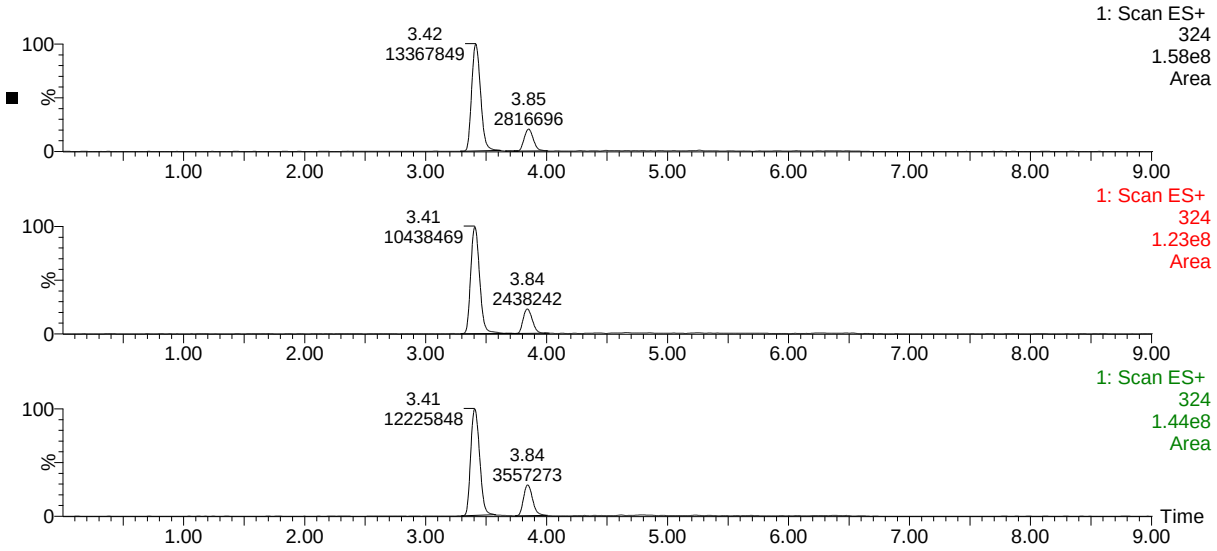


Figure S16. UPLC-MS analysis of the conversion of **1** (1.19 mM) by AmbDH3-CLEA after 8 min of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.41–3.42 min, t_R (**2**) = 3.84–3.85 min.

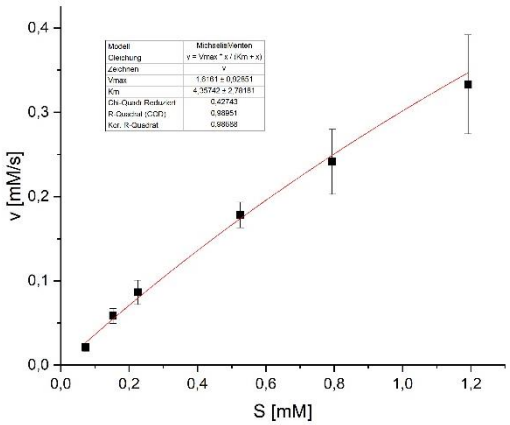


Figure S17 Kinetic analysis of AmbDH3-CLEA.

Table S3 Michaelis Menten kinetic parameters of AmbDH3-CLEA in comparison to free AmbDH3.

	AmbDH3-CLEA	**free AmbDH3**
k_{cat} [s^{-1}][a]	24.7 ± 14.2	230 ± 83[b]
K_m [mM][a]	4.4 ± 2.8	8.2 ± 3.1[b]
k_{cat}/K_m [s^{-1} mM^{-1}][a]	5.67 ± 4.87	28.2 ± 14.8[b]

[a]65.4 µM protein for AmbDH3-CLEA and 5 µM for AmbDH3. [b]As reported in [1].

Time course experiment with AmbDH3-CLEA

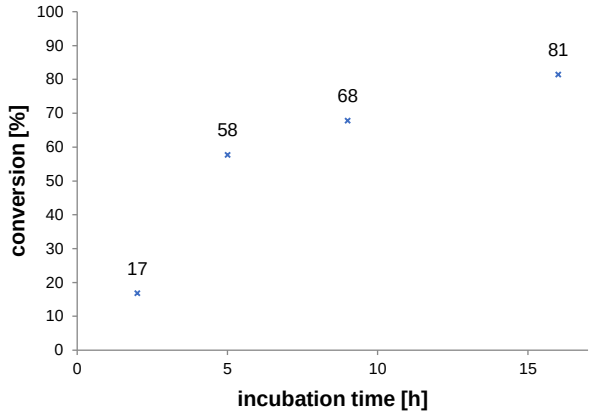


Figure S18. Time course experiment using AmbDH3-CLEA and **1**. Reaction conditions: 332 nmol (2 mm) of **1**, 1.9 x 10^{-3} u AmbDH3-CLEA, 30 °C, 16 h.

Various analytical scale conversions with AmbDH3-CLEA were set up and stopped after 2, 5, 9 or 16 h *via* addition of EtOAc (500 µL). The organic solvent was evaporated, the remaining solid was dissolved in MeOH and analysed *via* UPLC-MS.

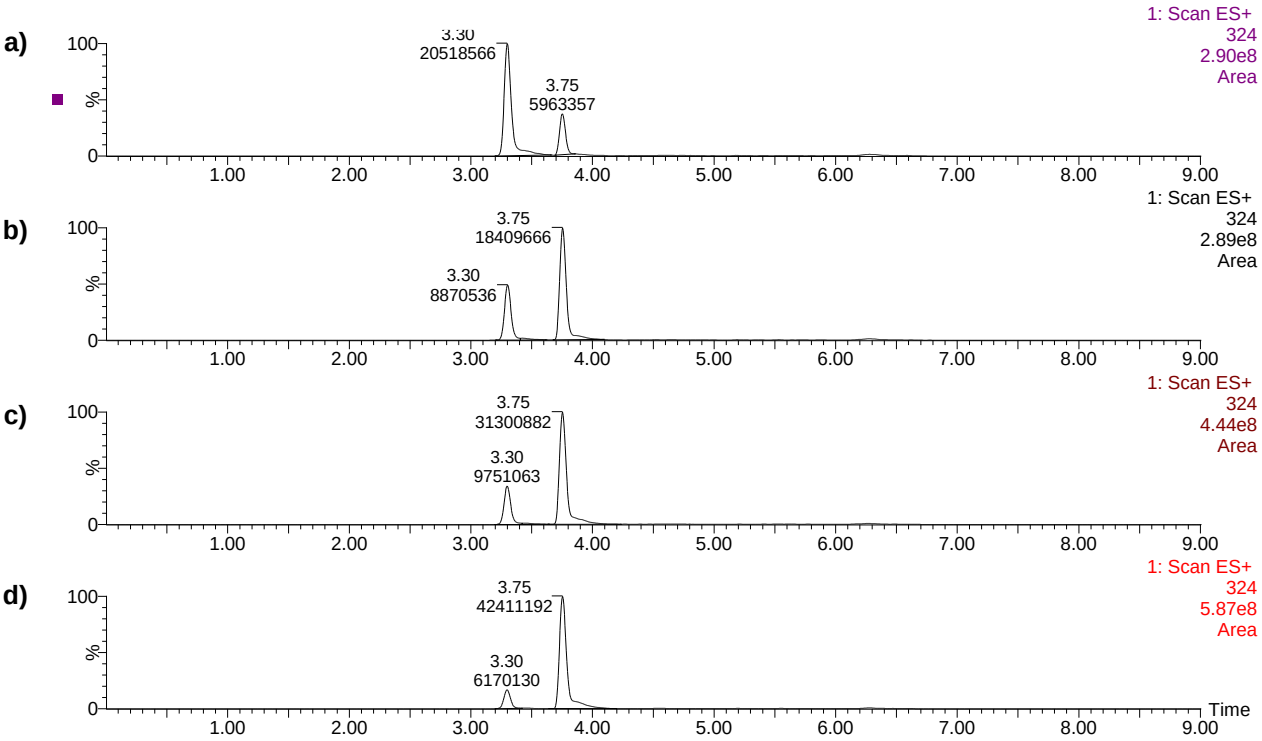


Figure S19. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after **a)** 2, **b)** 5, **c)** 9 and **d)** 16 h of incubation. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.30 min, t_R (**2**) = 3.75 min.

Stability of AmbDH3-CLEA

AmbDH3-CLEA was stored for 7 d at 4 °C or room temperature and afterwards used for analytical-scale enzymatic conversions. Alternatively, AmbDH3-CLEA was stored for 1 d at -20 °C or -80 °C, respectively. Enzymatic reactions were carried out after thawing of the pellet on ice with **1** (0.1 mg, final concentration 2 mM) at 30 °C and 300 rpm for 16 h as described above. After incubation, EtOAc (500 µL) was added and extracted. The organic solvent was evaporated, the residue dissolved in MeOH and analysed *via* UPLC-MS.

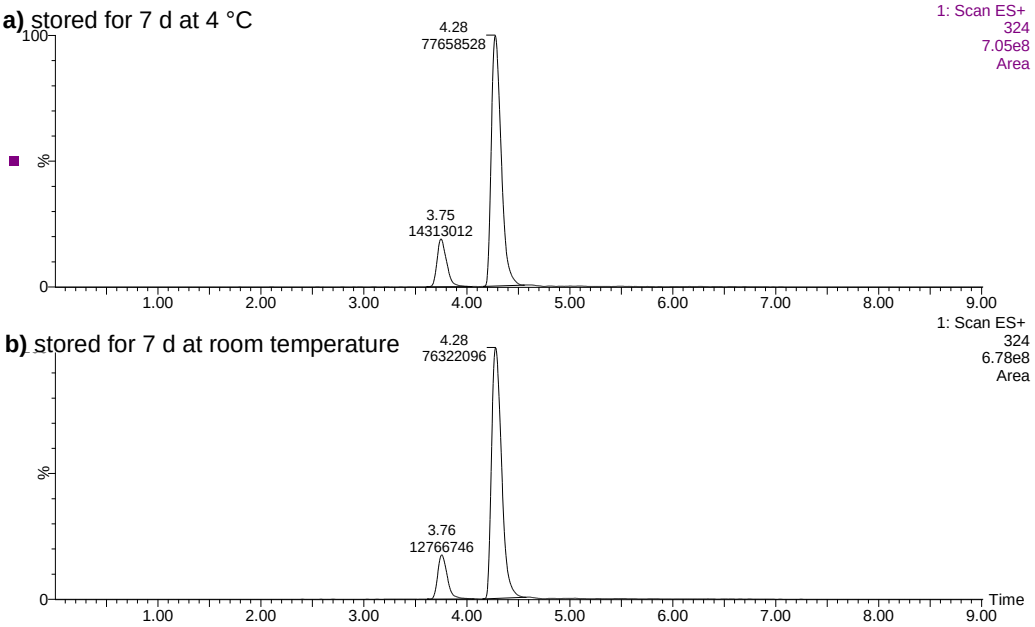


Figure S20. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after storage. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.75–3.76 min, t_R (**2**) = 4.28 min.

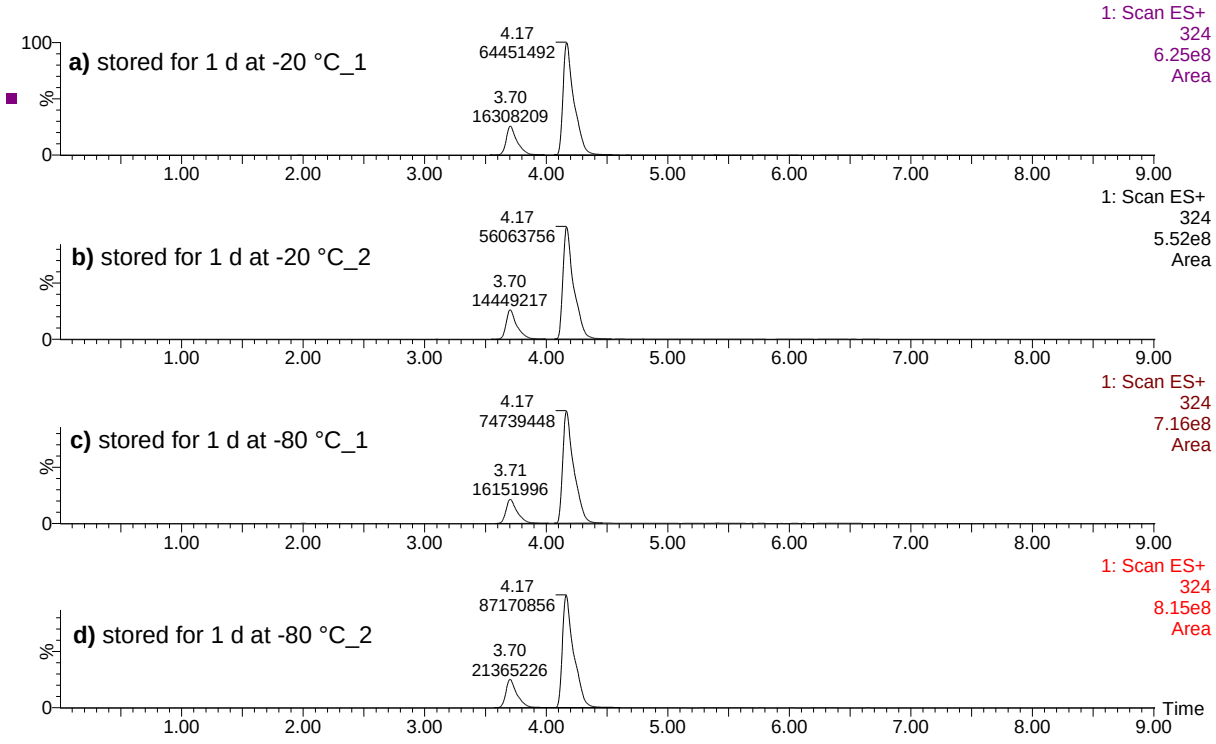


Figure S21. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after freeze-thawing. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.70–3.71 min, t_R (**2**) = 4.17 min.

Recycling of AmbDH3-CLEA

AmbDH3-CLEA was resuspended in sodium phosphate buffer (55 µL) and added to substrate **1** (0.1 mg, final concentration 2 mM). This procedure was carried out three times in total. The enzymatic reactions were incubated at 30 °C, 300 rpm for 16 h. Afterwards the reaction mixture was centrifuged (13000 g, 4 °C, 30 min), the supernatant was carefully moved to another vessel and extracted with EtOAc (500 µL). The organic solvent was evaporated, the remaining solid dissolved in MeOH and analysed *via* UPLC-MS. The remaining CLEA pellet was washed three times with sodium phosphate buffer (200 µL) and stored at 4 °C until usage. For use in the next reaction cycle the CLEA pellet was resuspended in sodium phosphate buffer (166 µL) and added to **1** (0.1 mg).

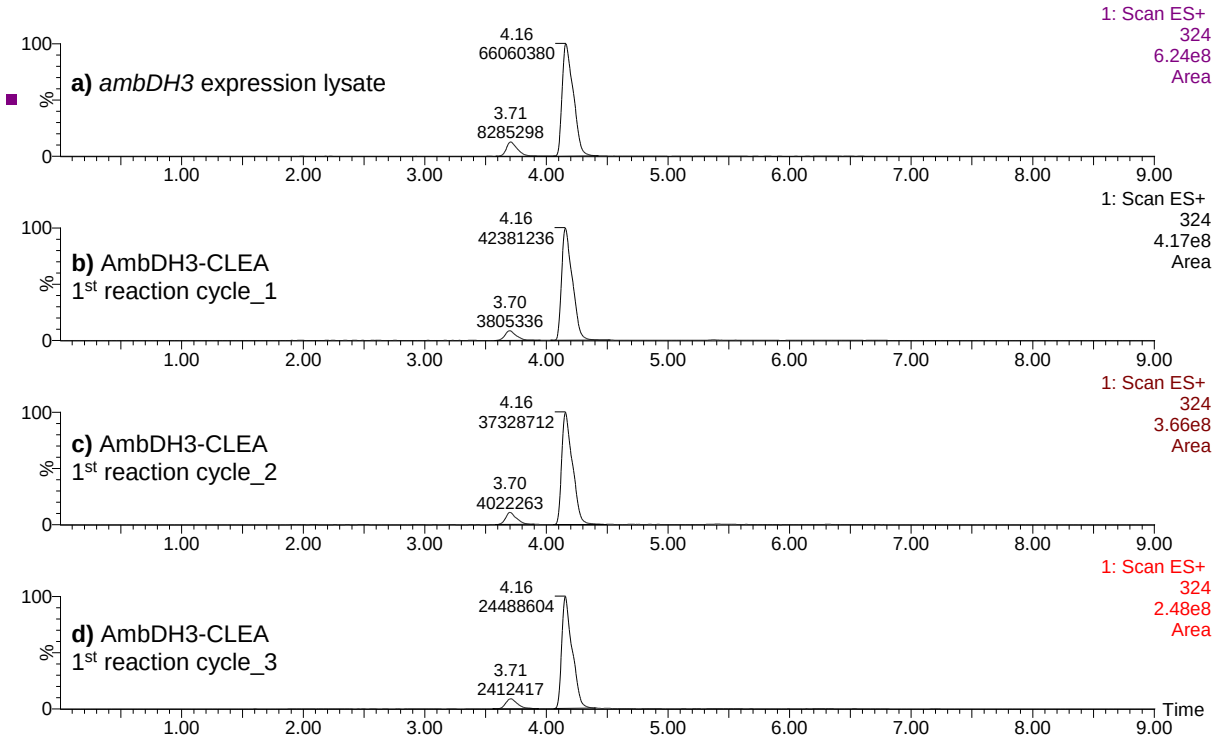


Figure S22. UPLC-MS analysis of the conversion of **1** by **a)** *ambDH3* expression lysate and **b)** AmbDH3-CLEA after one cycle of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.70–3.71 min, t_R (**2**) = 4.16 min.

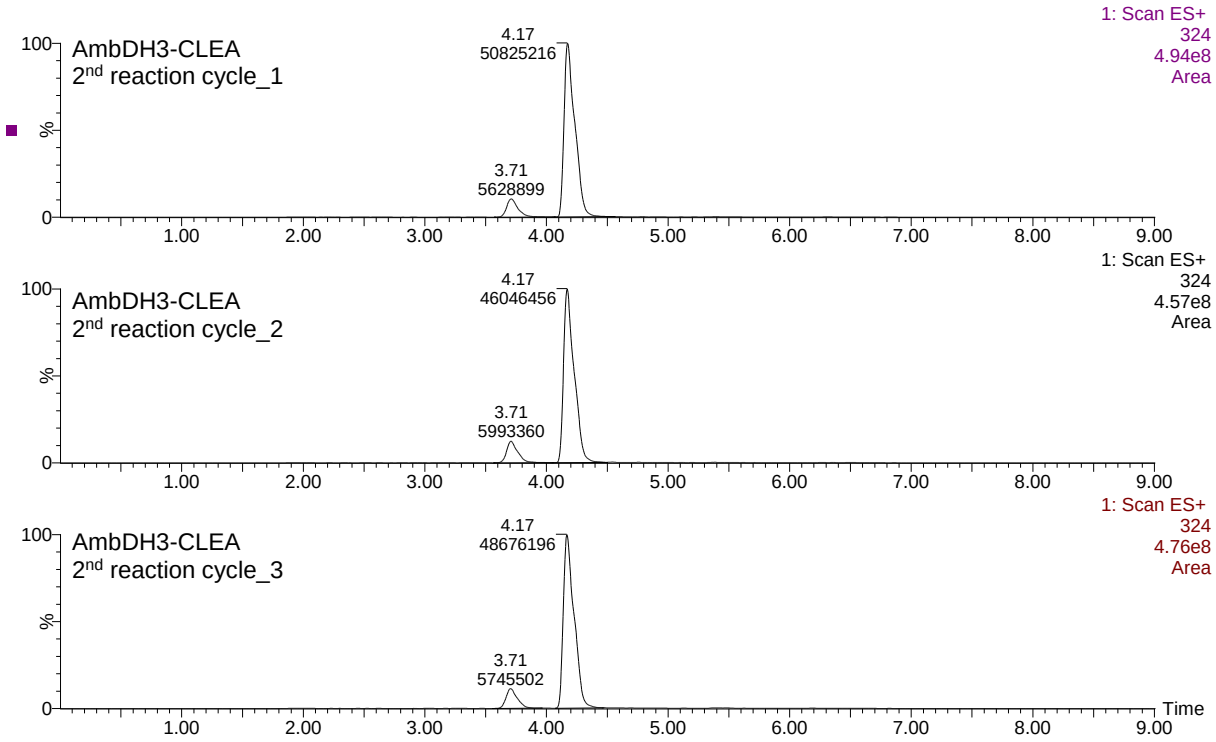


Figure S23. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after two cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.70–3.71 min, t_R (**2**) = 4.17 min.

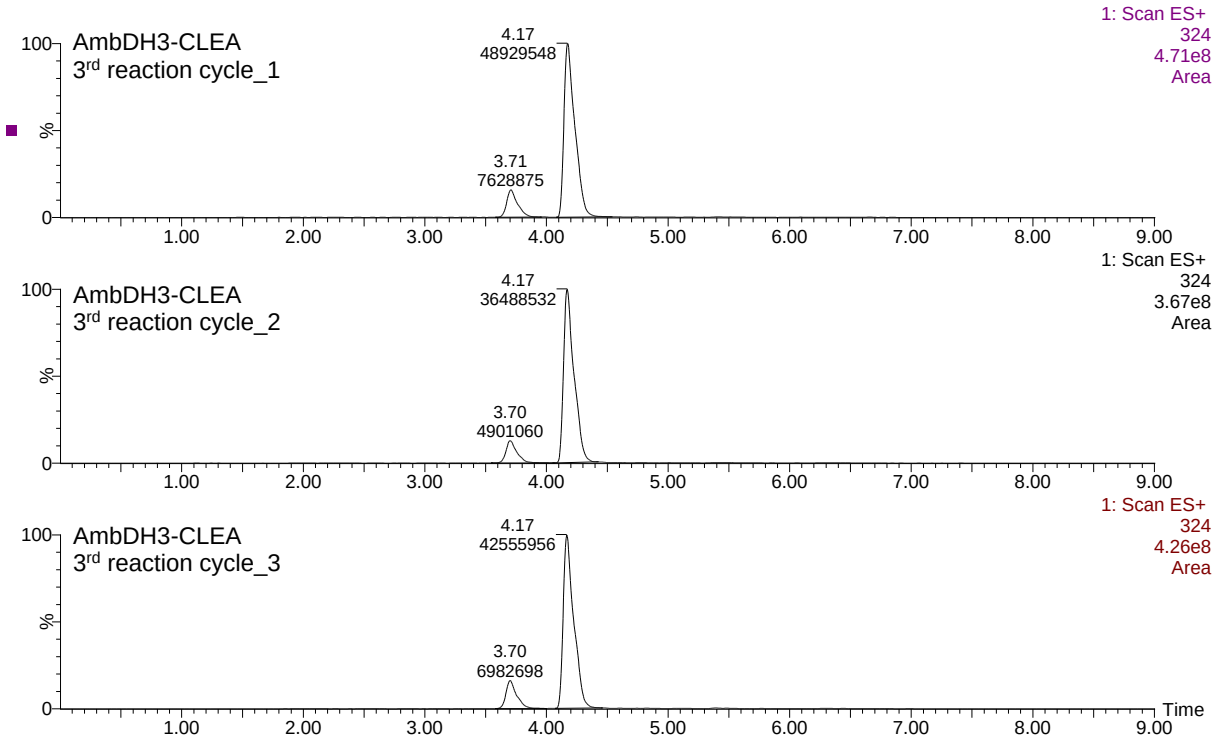


Figure S24. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after three cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.70–3.71 min, t_R (**2**) = 4.17 min.

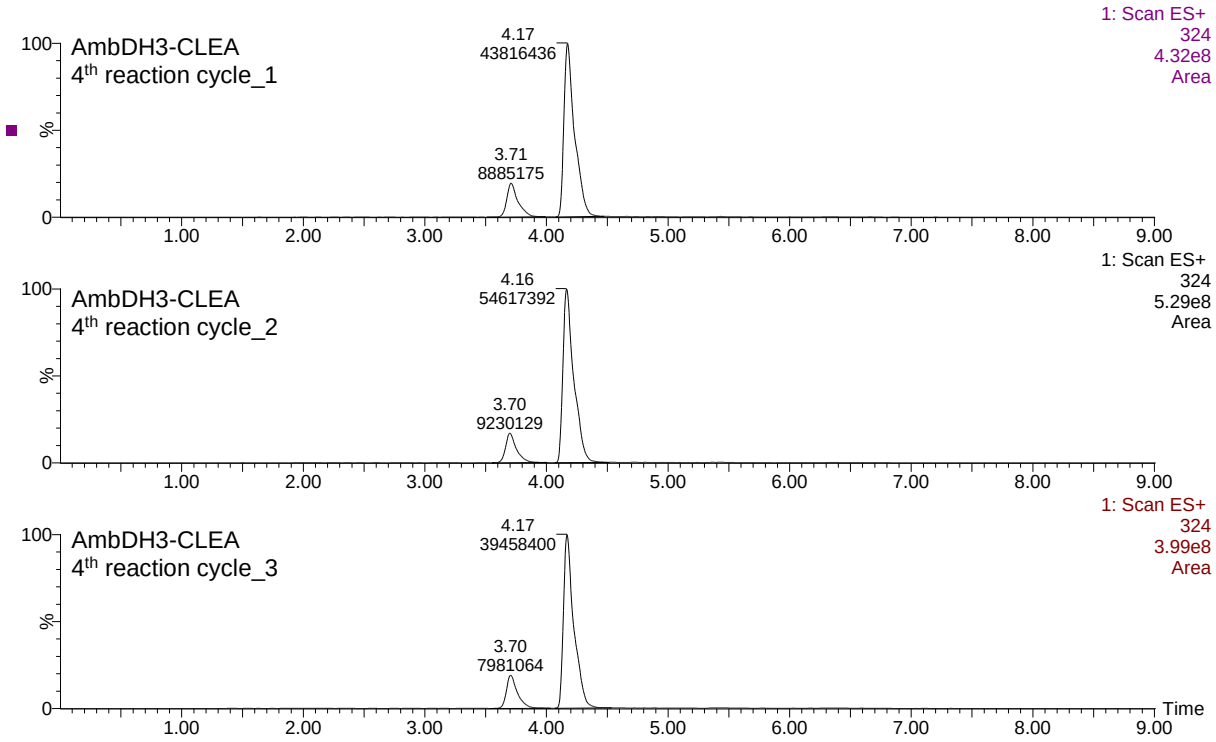


Figure S25. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after four cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.70–3.71 min, t_R (**2**) = 4.16–4.17 min.

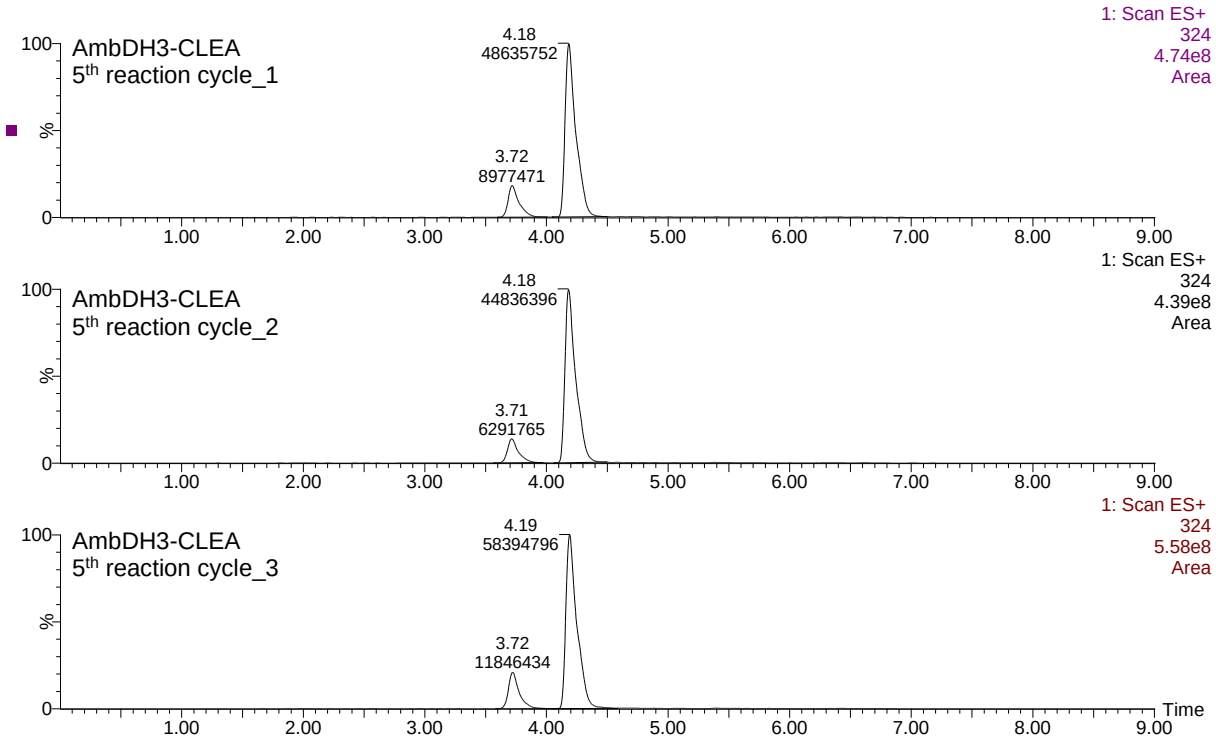


Figure S26. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after five cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.71–3.72 min, t_R (**2**) = 4.18–4.19 min.

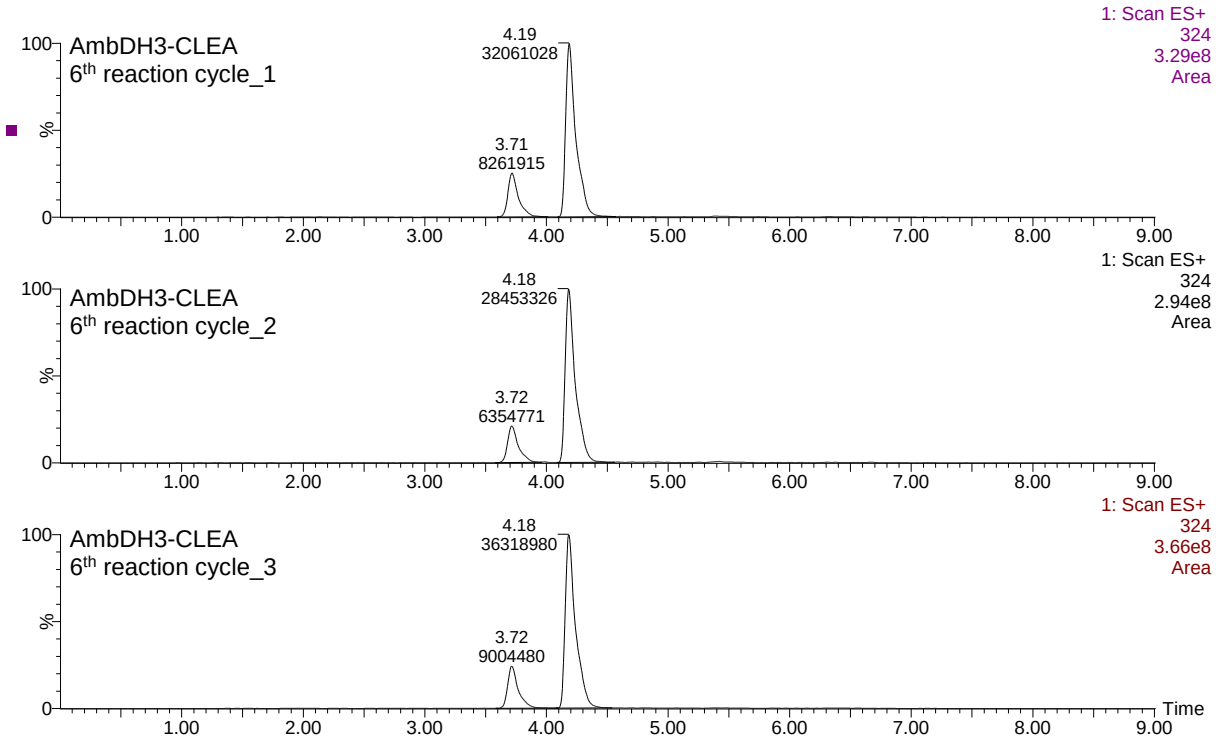


Figure S27. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after six cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.71–3.72 min, t_R (**2**) = 4.18–4.19 min.

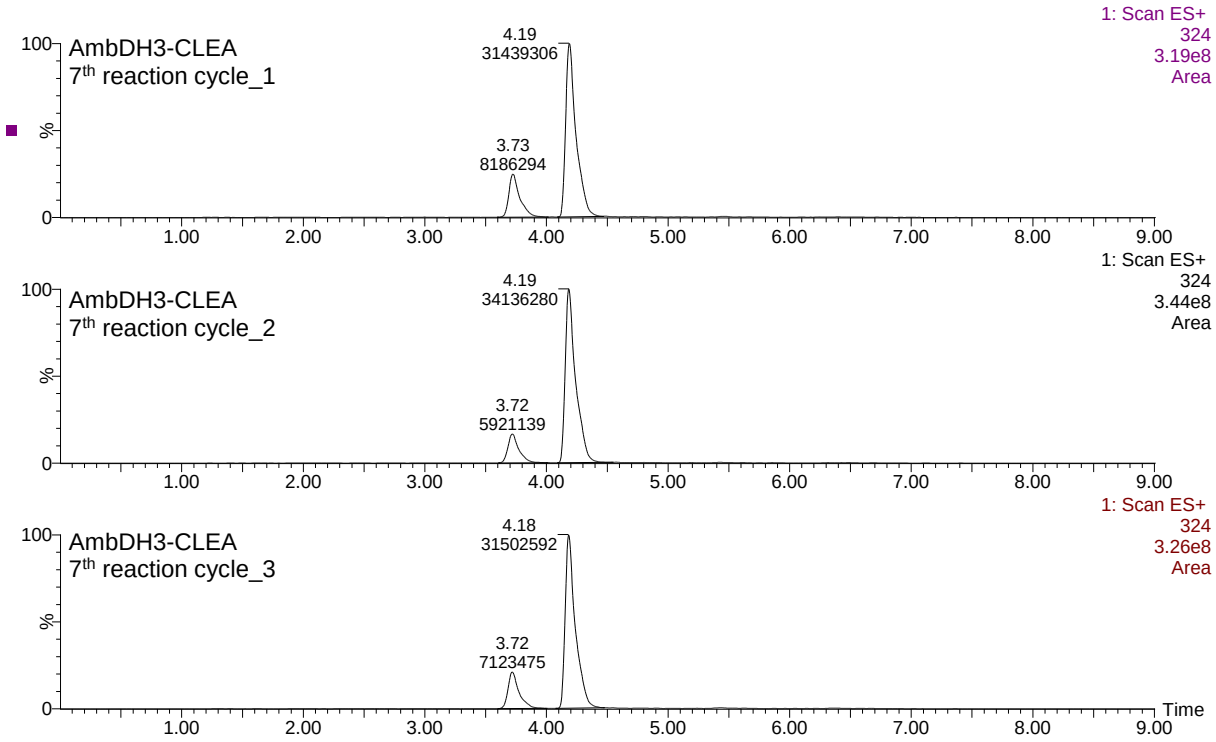


Figure S28. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after seven cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.72–3.73 min, t_R (**2**) = 4.18–4.19 min.

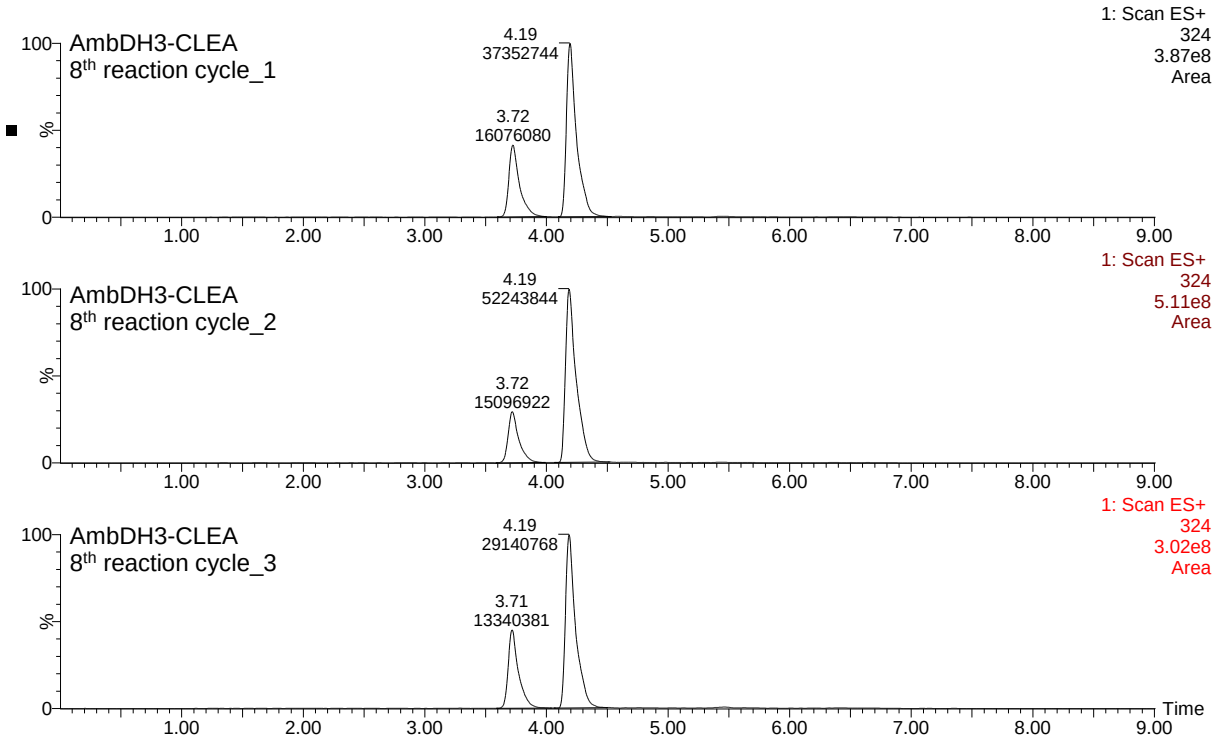


Figure S29. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after eight cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.71–3.72 min, t_R (**2**) = 4.19 min.

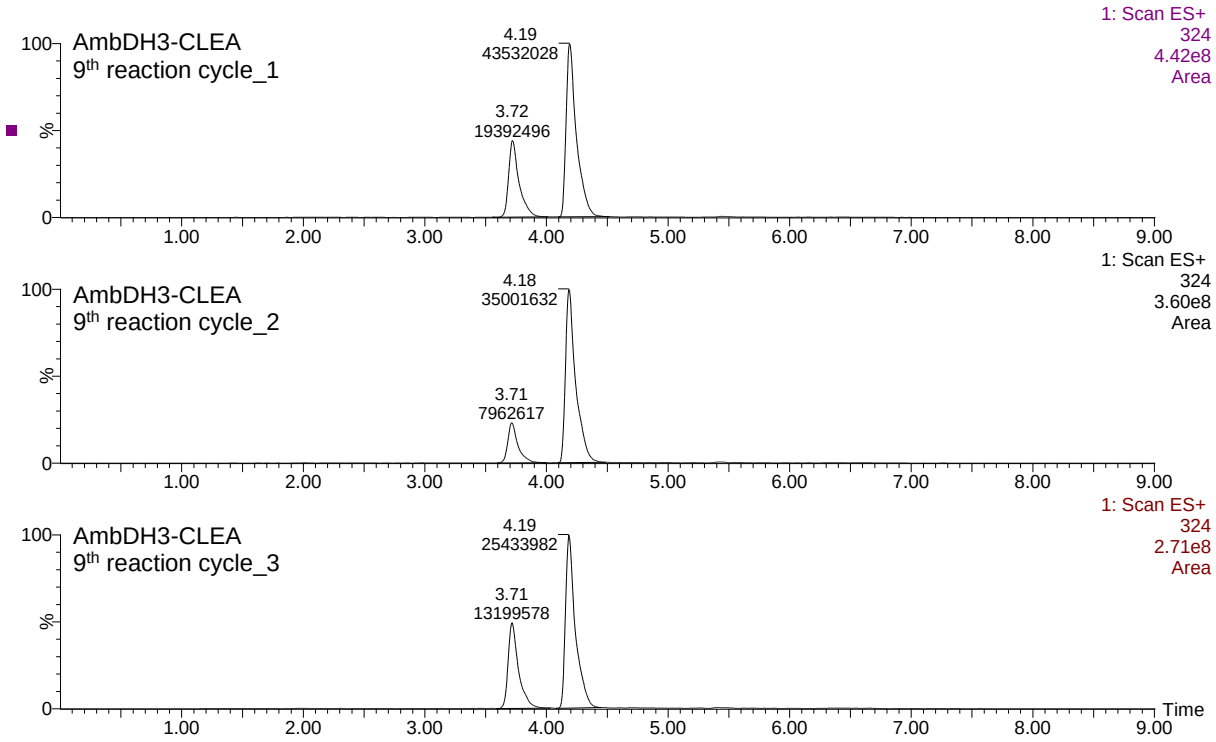


Figure S30. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after nine cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.71–3.72 min, t_R (**2**) = 4.18–4.19 min.

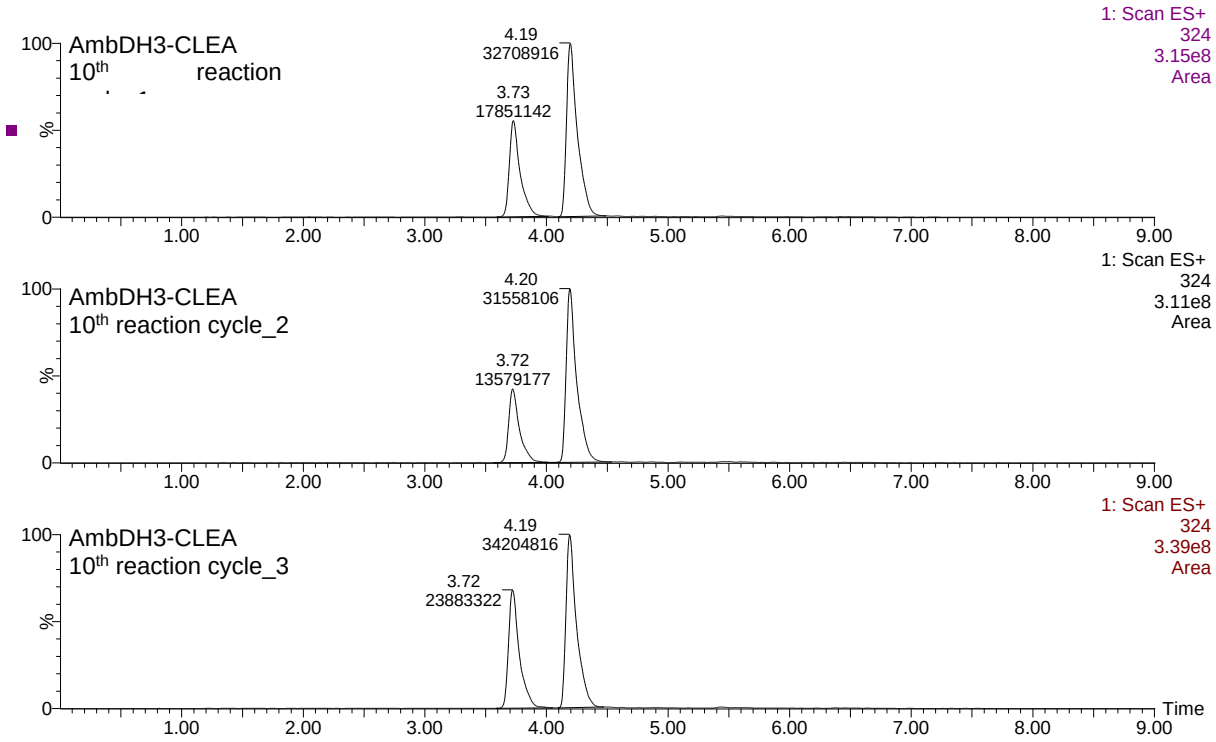
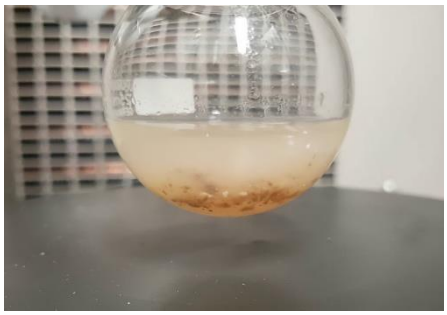


Figure S31. UPLC-MS analysis of the conversion of **1** by AmbDH3-CLEA after ten cycles of incubation carried out as a triplicate. M (**1**) = 324, M (**2**) = 324, t_R (**1**) = 3.72 min, t_R (**2**) = 4.19–4.20 min.

Semipreparative scale conversions with AmbDH3-CLEA



For semipreparative scale conversions, **1** (10 mg, final concentration 4 mM) was stirred at 300 rpm and 30 °C with the corresponding amount of the AmbDH3-CLEA suspension in a 25 mL flask. After 16 h, the solution was transferred to a 15 mL tube. The flask was washed twice with sodium phosphate buffer (2 mL), the suspension added to the tube and centrifuged (10000 g, 4 °C, 30 min). The supernatant was transferred to a 50 mL tube. The residual CLEA pellet was washed three times with sodium phosphate buffer (5 mL) and centrifuged (10000 g, 4 °C, 15 min). The remaining CLEA pellet was stored at 4 °C until usage in the next reaction cycle. The supernatant and washing fractions were extracted three times with EtOAc (10 or 5 mL), respectively, and centrifuged for better phase separation (5000 g, 4 °C, 10 min). The organic solvent was evaporated, the obtained solids were dissolved in MeOH and analysed *via* UPLC-MS or NMR spectroscopy. After purification by flash chromatography on silica gel (hexane:Et_2O:EtOAc / 1:0:0→4:1:0→0:0:1) the pooled reaction product (47 mg, 157 µmol, 94%) was obtained as a colourless solid.

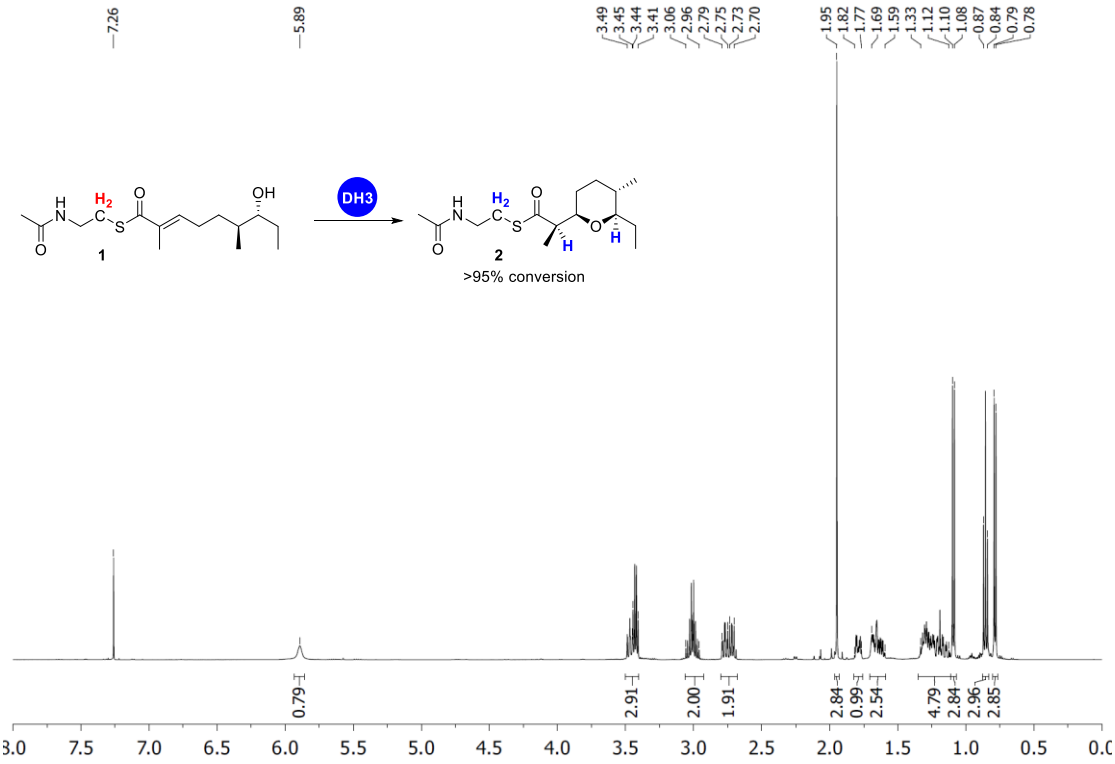


Figure S32. ^{1}H NMR analysis of the conversion of **1** by AmbDH3-CLEA on the semipreparative scale, 1st reaction cycle. The conversion was determined from the integrals corresponding to the protons highlighted in red and blue. δ(CH_2-S, CH_2-S) = 2.96–3.06 ppm; δ(2-H, 7-H) = 2.70–2.79 ppm.

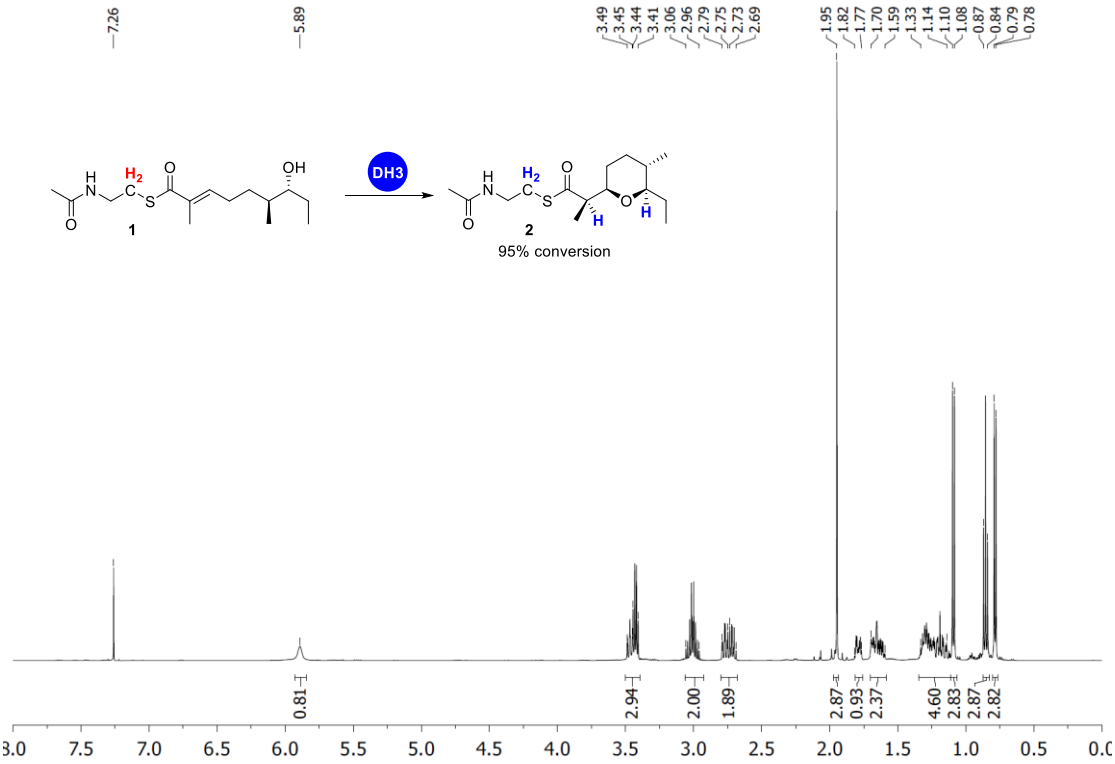


Figure S33. ^{1}H NMR analysis of the conversion of **1** by AmbDH3-CLEA on the semipreparative scale, 2nd reaction cycle. The conversion was determined from the integrals corresponding to the protons highlighted in red and blue. δ(CH_2-S, CH_2-S) = 2.96–3.06 ppm; δ(2-H, 7-H) = 2.69–2.79 ppm.

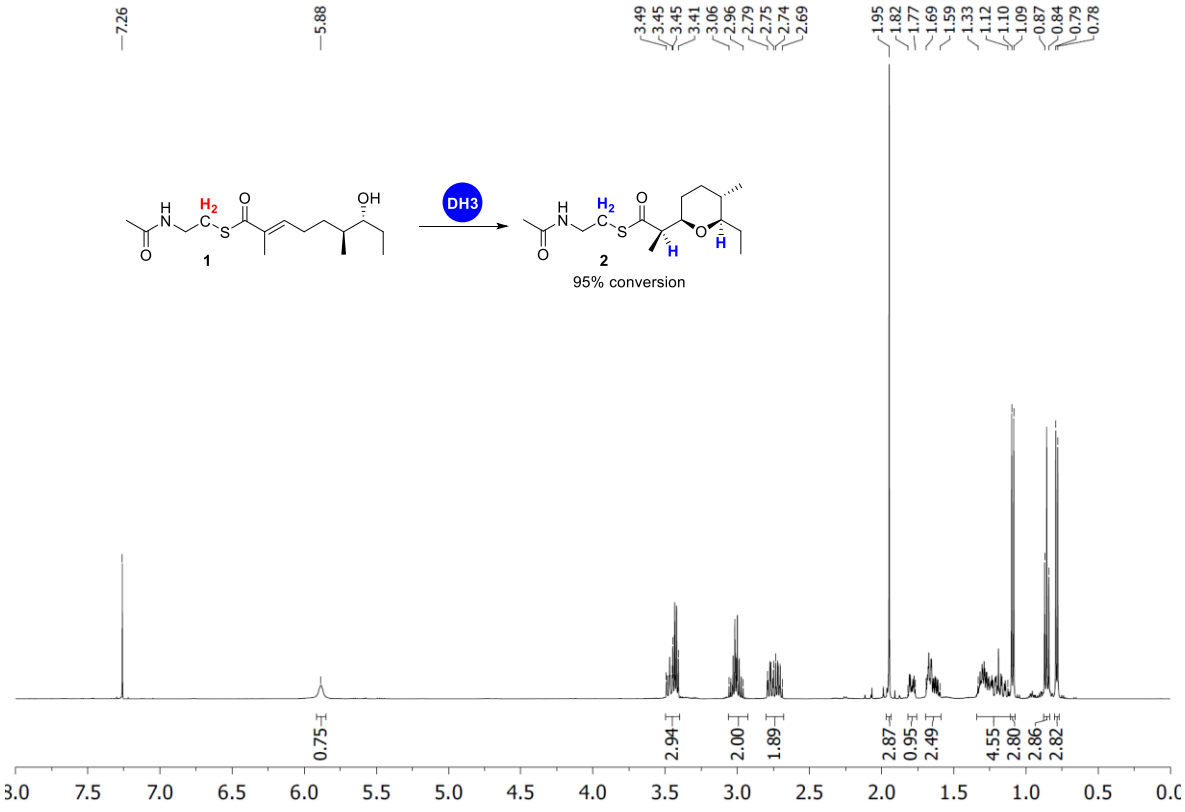


Figure S34. ^{1}H NMR analysis of the conversion of **1** by AmbDH3-CLEA on the semipreparative scale, 3rd reaction cycle. The conversion was determined from the integrals corresponding to the protons highlighted in red and blue. δ(CH_2-S, CH_2-S) = 2.96–3.06 ppm; δ(2-H, 7-H) = 2.69–2.79 ppm.

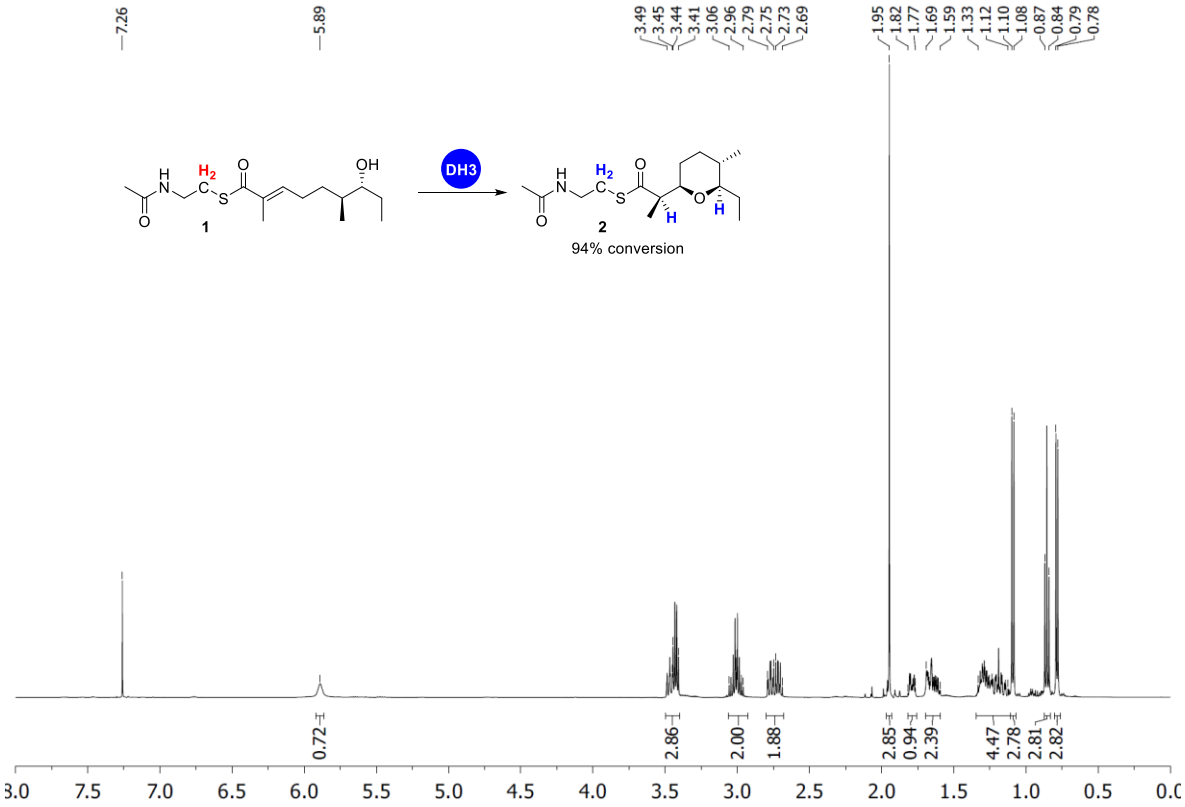


Figure S35. ^{1}H NMR analysis of the conversion of **1** by AmbDH3-CLEA on the semipreparative scale, 4th reaction cycle. The conversion was determined from the integrals corresponding to the protons highlighted in red and blue. δ(CH_2-S, CH_2-S) = 2.96–3.06 ppm; δ(2-H, 7-H) = 2.69–2.79 ppm.

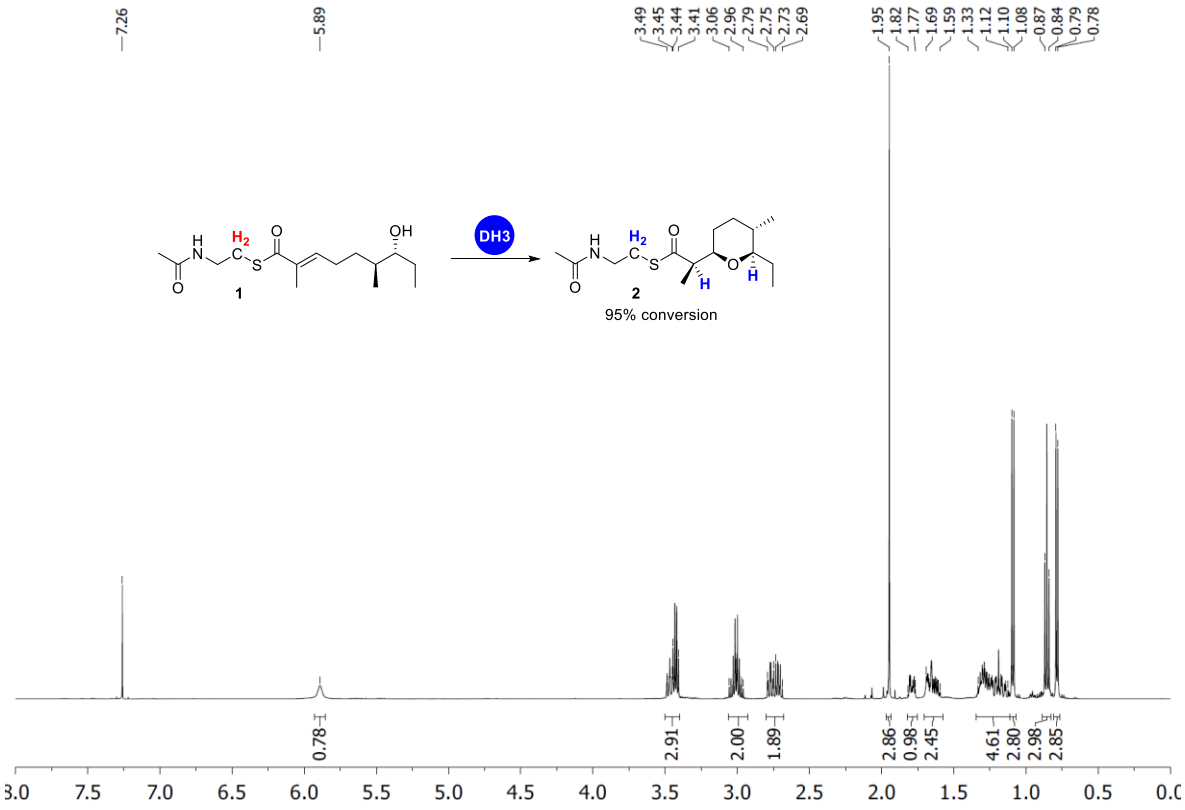
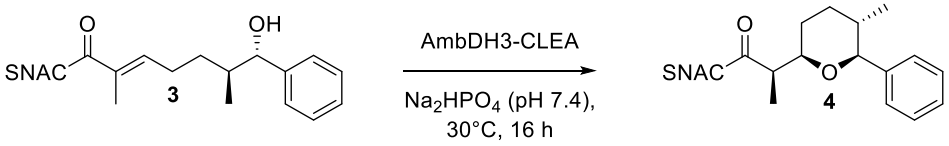


Figure S36. ^{1}H NMR analysis of the conversion of **1** by AmbDH3-CLEA on the semipreparative scale, 5th reaction cycle. The conversion was determined from the integrals corresponding to the protons highlighted in red and blue. δ(CH_2-S, CH_2-S) = 2.96–3.06 ppm; δ(2-H, 7-H) = 2.69–2.79 ppm.

Substrate tolerance and kinetic resolution

S-(2-Acetamidoethyl) (*R*)-2-((2R,5S,6S)-5-methyl-6-phenyltetrahydro-2*H*-pyran-2-yl)propanethioate (4)



Following the general method for semipreparative scale conversions with AmbDH3-CLEA, alcohol **3** (10 mg, 28.6 µmol) was reacted to yield THP **4** (4.1 mg, 11.7 µmol, 41%) after purification by flash chromatography on silica gel (EtOAc:CH_2Cl_2 / 1:1).

R_f = 0.33 (EtOAc:CH_2Cl_2 / 1:1); **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 7.33-7.29 (m, 4H, C*H*$_{Ar}$), 7.27-7.24 (m, 1H, C*H*$_{Ar}$), 5.23 (bs, 1H, CH_2N*H*), 3.85 (d, *J* = 9.8 Hz, 1H, OC*H*C), 3.70-3.66 (m, 1H, CCHC*H*O), 3.20-3.16 (m, 2H, C*H*$_2$NH), 3.10-3.05 (m, 1H, 1×C*H*$_2$S), 2.85-2.75 (m, 2H, 1×C*H*$_2$S, CC*H*CH_3), 1.97-1.93 (m, 1H, 1×C*H*$_2$CHCH$_3$), 1.81-1.78 (m, 1H, 1×CCHCHC*H*$_2$), 1.65 (s, 3H, OCC*H*$_3$), 1.62-1.56 (m, 1H, CH_2C*H*CH_3), 1.45-1.32 (m, 2H, 1×CCHCHC*H*$_2$, 1×C*H*$_2$CHCH$_3$), 1.14 (d, *J* = 7.0 Hz, 3H, CCHC*H*$_3$), 0.65 (d, *J* = 6.6 Hz, 3H, CH_2CHC*H*$_3$).

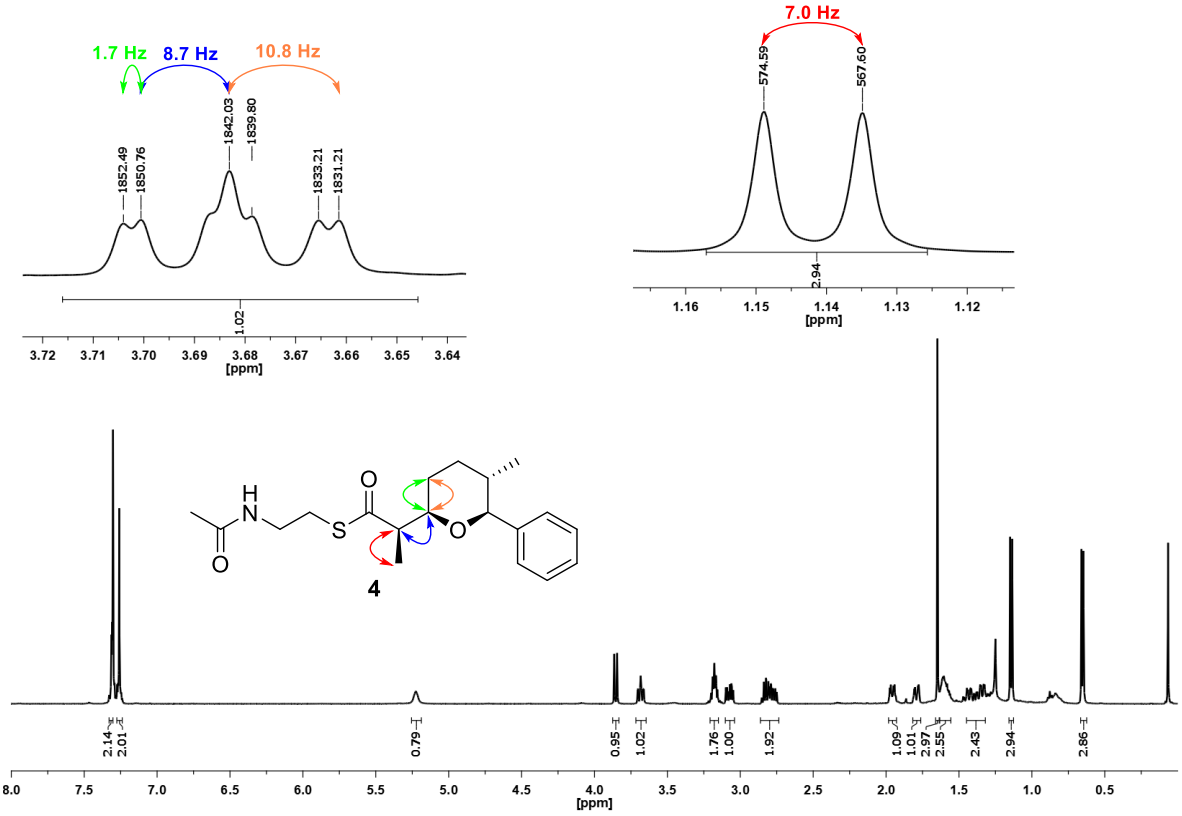


Figure S37. ^{1}H NMR analysis of the purified assay product **4** ($CDCl_3$, 500 MHz). The shift values for 7-H and 3-H (δ=3.8 and 3.7 ppm) indicate a 2,6-*cis* configuration of the THP.[6] The vicinal coupling constant $^3J_{2H\text{-}3H}$ of 8.7 Hz is consistent with the shown configuration. The configurational assignment is described in reference [1].

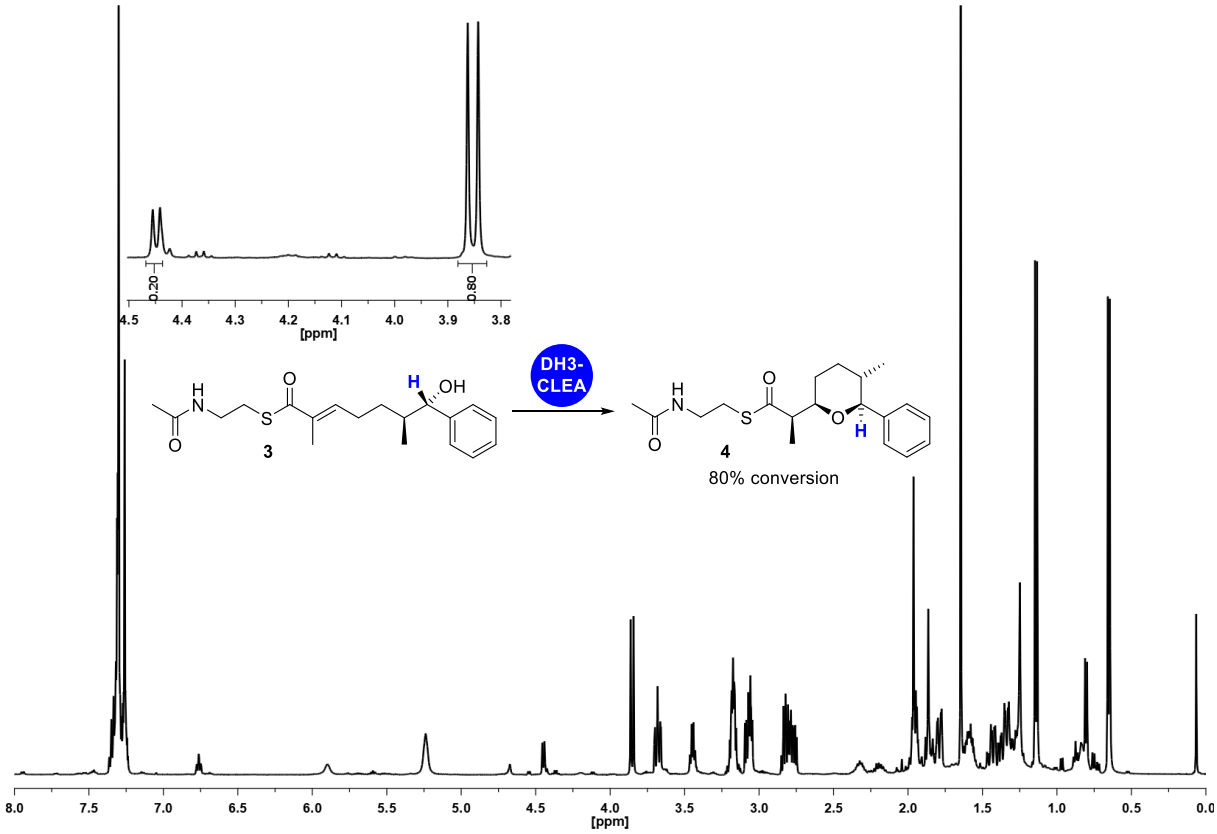
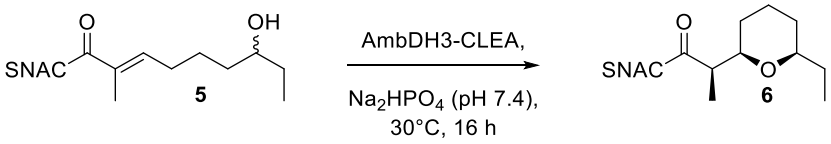


Figure S38. a) ^{1}H NMR analysis of the conversion experiment of AmbDH3-CLEA and **3** ($CDCl_3$, 500 MHz). The conversion was determined from the integrals corresponding to the protons highlighted in blue. No conversion was observed in an enzyme-free incubation under otherwise similar conditions.[1]

_S_-(2-Acetamidoethyl) (_R_)-2-((2R,6R)-6-ethyltetrahydro-2_H_-pyran-2-yl)propanthioate (6)



Following the general method for semipreparative scale conversions with AmbDH3-CLEA, alcohol mixture ***rac*-5** (10 mg, 34.8 µmol) was reacted to yield a mixture of THP **(*R*)-6** and ***rac*-5** (2 mg, 6.96 µmol, 20%) after purification by flash chromatography on silica gel (EtOAc).

R_f = 0.45 (EtOAc); **^{1}H NMR** (500 MHz, $CDCl_3$): δ [ppm] = 5.85 (bs, 1H, CH_2NH), 3.52 (ddd, J = 11.0, 9.0, 1.9 Hz, 1H, $OCHCH$), 3.44 (q, J = 6.1 Hz, 2H, CH_2NH), 3.17-3.11 (m, 1H, $OCHCH_2CH_3$), 3.08-2.96 (m, 2H, SCH_2), 2.76-2.70 (m, $CCHCH_3$), 1.95 (s, 3H, $OCCH_3$), 1.88-1.84 (m, 1H, $1\times CHCH_2CH_2$), 1.66-1.64 (m, 1H, $1\times CHCHCH_2$), 1.50-1.37 (m, 4H, $1\times CH_3CH_2CHCH_2$, CH_2CH_3, $1\times CHCH_2CH_2$), 1.20-1.10 (m, 2H, $1\times CHCHCH_2$, $1\times CH_3CH_2CHCH_2$), 1.10 (d, J = 7.0 Hz, 3H, $CCHCH_3$), 0.86 (t, J = 7.4 Hz, 3H, CH_2CH_3).

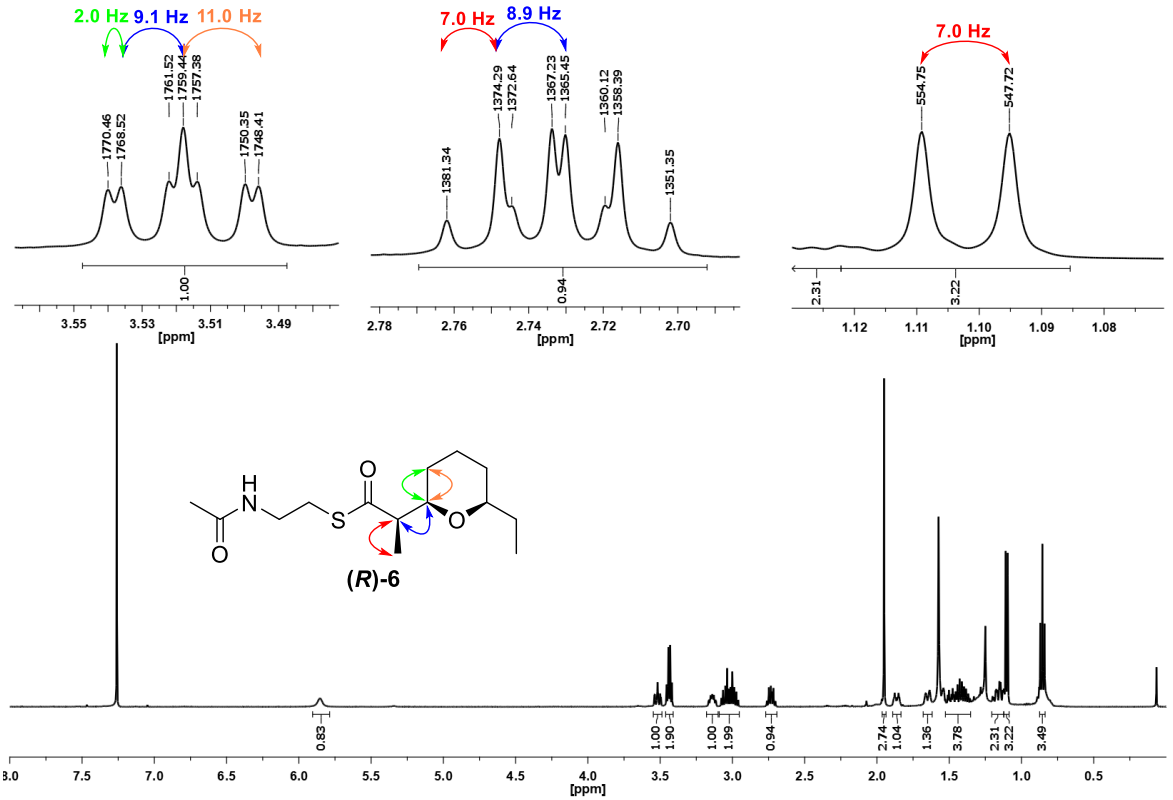


Figure S39. ^{1}H NMR analysis of the purified assay product **(*R*)-6** ($CDCl_3$, 500 MHz). Due to the presence of the phenyl ring, the multipletts for 7-H and 3-H (δ=3.8 and 3.7 ppm) are shifted downfield. These values indicate a 2,6-*cis* configuration of the THP.[21] The vicinal coupling constant $^3J_{2H\text{-}3H}$ of 8.7 Hz is consistent with the shown configuration

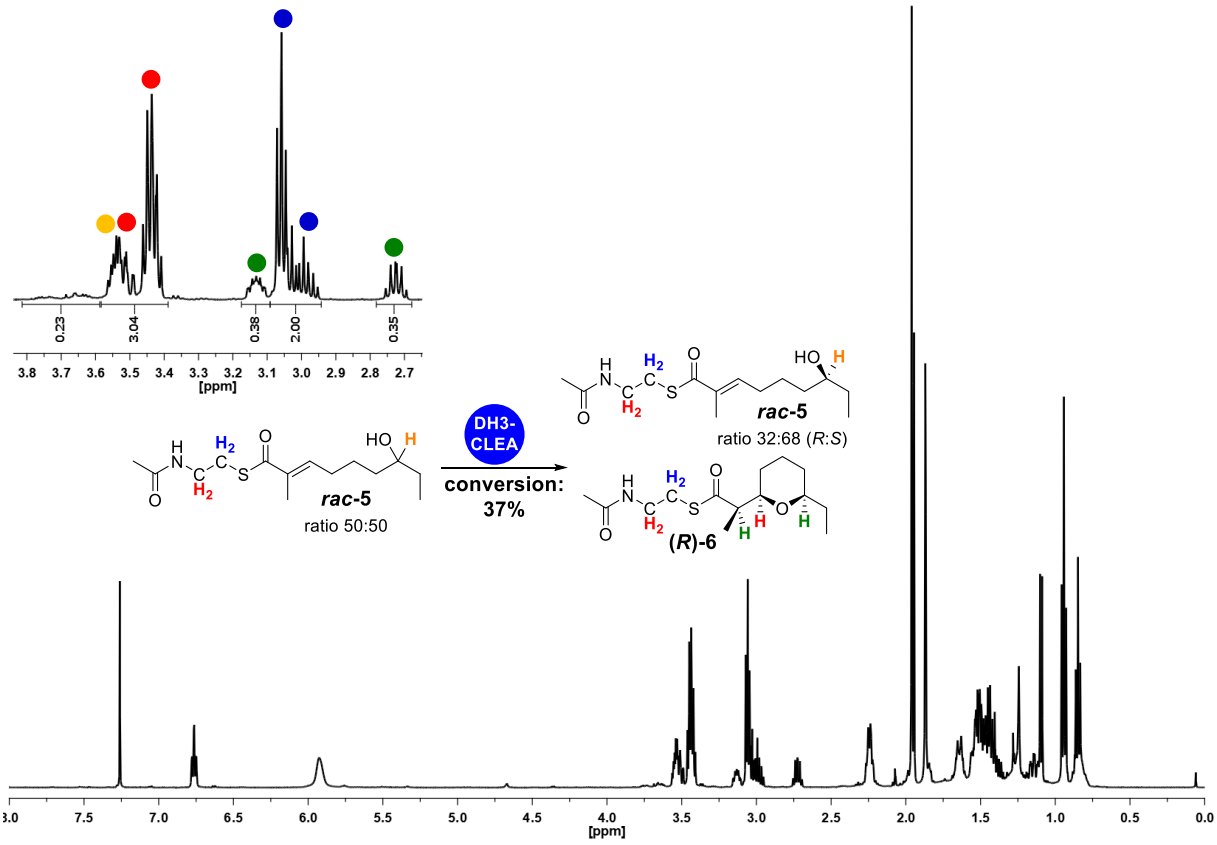
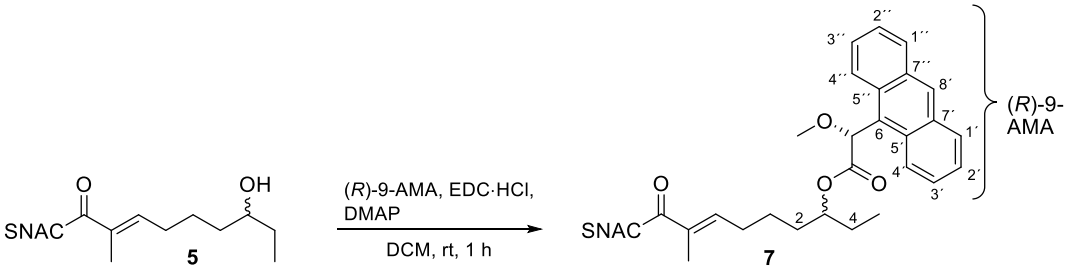
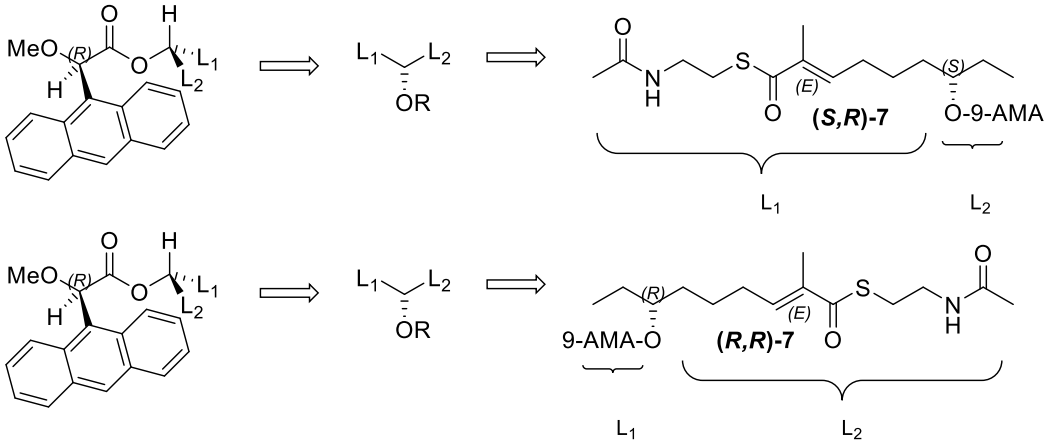


Figure S40. ^{1}H NMR analysis of the conversion experiment of AmbDH3-CLEA and **5** ($CDCl_3$, 500 MHz). The conversion was determined from the integrals corresponding to the protons highlighted in blue and green. For 2,6-*trans*-THPs, δ(3-H) and δ(7-H) would be expected to be at 3.6–4.0 ppm. Integration of this region showed only low overall intensity and no spin systems that would fit to the literature-described 2,6-*trans*-THPs.[7] No conversion was observed in an enzyme-free incubation under otherwise similar conditions.[1]

(*E*)-9-((2-Acetamidoethyl)thio)-8-methyl-9-oxonon-7-en-3-yl (2*R*)-2-(anthracen-9-yl)-2-methoxyacetat (*rac*-XY)[8]



(*R*)-9-AMA (9.2 mg, 34.8 µmol, 2.5 equiv.) was treated with alcohol **5** (4 mg, 13.9 µmol, 1.0 equiv.), EDC·HCl (6.6 mg, 34.8 µmol, 2.5 equiv.) and DMAP (1 mg, 8.9 µmol, 0.7 equiv.) in abs. CH_2Cl_2 (0.278 mL). After 1 h of stirring, H_2O was added to the mixture and the aqueous phase was extracted with CH_2Cl_2 (2 ×). The combined organic phases were washed with 1 M HCl (2 ×), H_2O, sat. $NaHCO_3$ aq. (2 ×) and H_2O, dried over $MgSO_4$, filtrated and concentrated *in vacuo* to give a yellow oil. The esterification proceeded with quantitative conversion and yielded the (*S,R*):(*R,R*) diastereomers of **7** in a 68:32 ratio.



(*S,R*)-7

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 8.57 (d, J = 8.8 Hz, 2H, 3-, 3´-C$H_{anthr.}$), 8.48 (s, 1H, 8-C$H_{anthr.}$), 8.03–7.99 (m, 2H, 6-, 6´-C$H_{anthr.}$), 7.56-7.51 (m, 2H, 4-, 4´-C$H_{anthr.}$), 7.50-7.44 (m, 2H, 5-, 5´-C$H_{anthr.}$), 6.66 (td, J = 7.3, 1.2 Hz, 1H, CHOCH$_3$), 6.28 (s, 1H, CH_3CCH), 5.93 (bs, 1H, NH), 4.85-4.81 (m, 1H, CH_3CH_2CHO), 3.48-3.43 (m, 2H, NHCH_2), 3.41 (s, 3H, CHOCH_3), 3.09-3.06 (m, 2H, SCH_2), 2.19-2.12 (m, 2H, CH_3CCHCH_2), 1.96 (s, 3H, NHCOCH_3), 1.83 (s, 3H, CH_3CCH), 1.50-1.37 (m, 4H, CCHCH$_2$CH_2, CCHCH$_2$CH$_2$CH_2), 1.19-1.02 (m, 2H, CH_3CH_2), 0.12 (t, J = 7.4 Hz, 3H, CH_3CH$_2$).

(*R,R*)-7

^{1}H NMR (500 MHz, $CDCl_3$): δ [ppm] = 8.57 (d, J = 8.8 Hz, 2H, 3-, 3´-C$H_{anthr.}$), 8.48 (s, 1H, 8-C$H_{anthr.}$), 8.03–7.99 (m, 2H, 6-, 6´-C$H_{anthr.}$), 7.56-7.51 (m, 2H, 4-, 4´-C$H_{anthr.}$), 7.50-7.44 (m, 2H, 5-, 5´-C$H_{anthr.}$), 6.28 (s, 1H, CH_3CCH), 6.12 (td, J = 7.3, 1.4 Hz, 1H, CHOCH$_3$), 5.93 (bs, s, 1H, NH), 4.85-4.81 (m, 1H, CH_3CH_2CHO), 3.48-3.43 (m, 2H, NHCH_2), 3.44 (s, 3H, CHOCH_3), 3.09-3.06 (m, 2H, SCH_2), 1.98 (s, 3H, NHCOCH_3), 1.59 (s, 3H, CH_3CCH), 1.50-1.37 (m, 6H, CCHCH_2, CCHCH$_2$CH_2, CH_3CH_2), 1.19–1.02 (m, 2H, CCHCH$_2$CH$_2$CH_2), 0.82 (t, J = 7.3 Hz, 3H, CH_3CH$_2$).

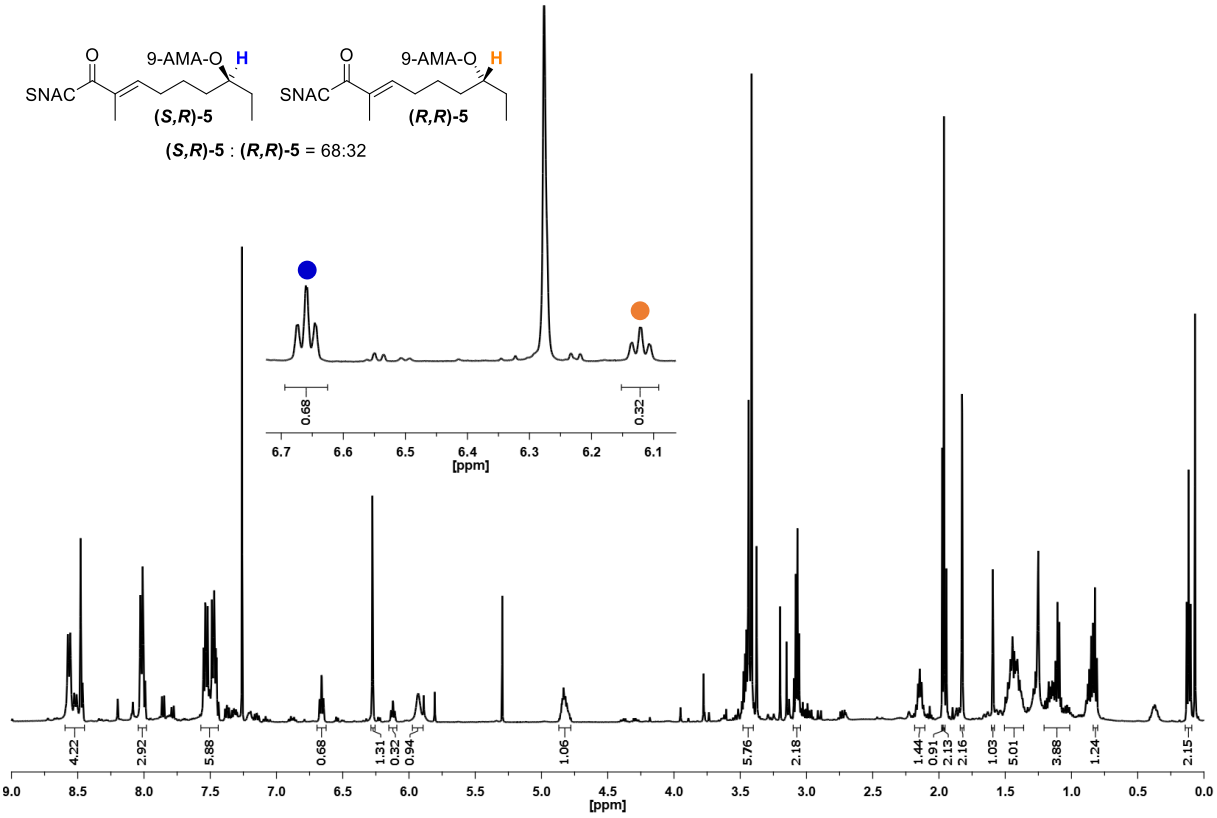
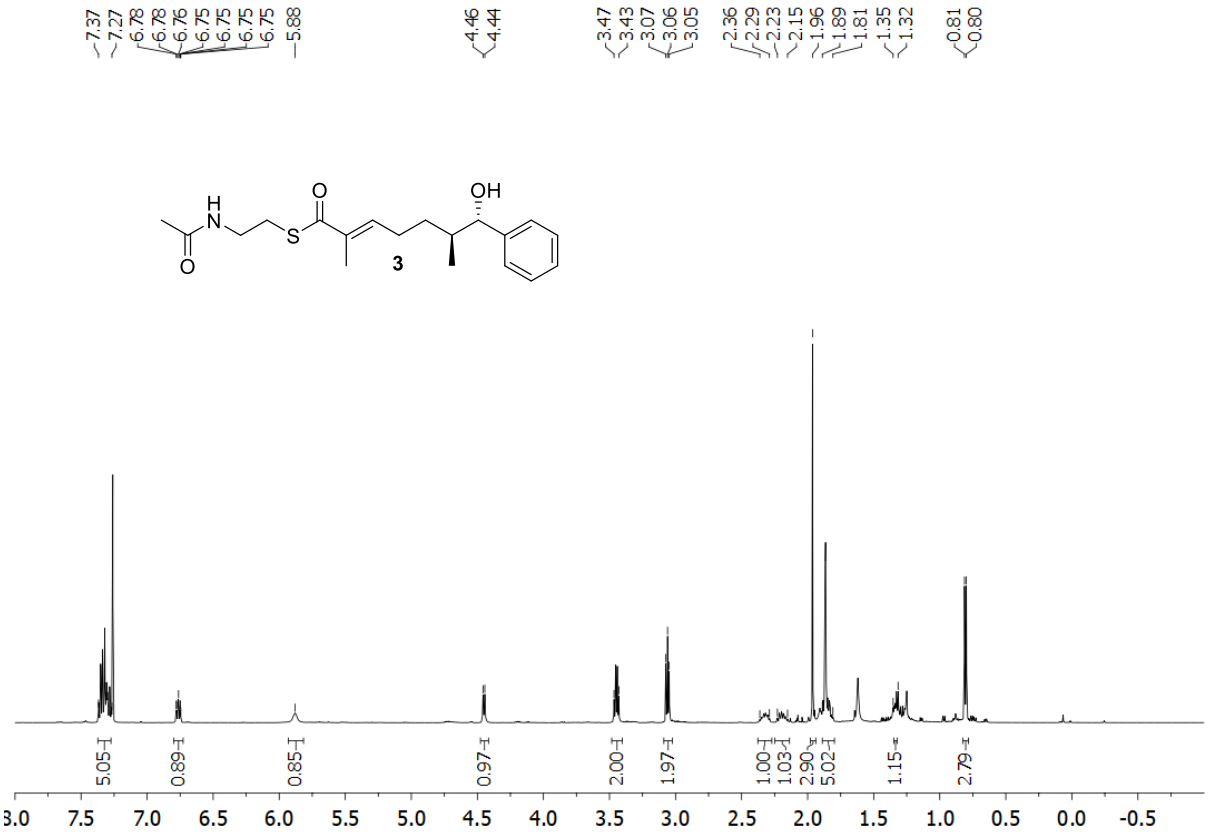
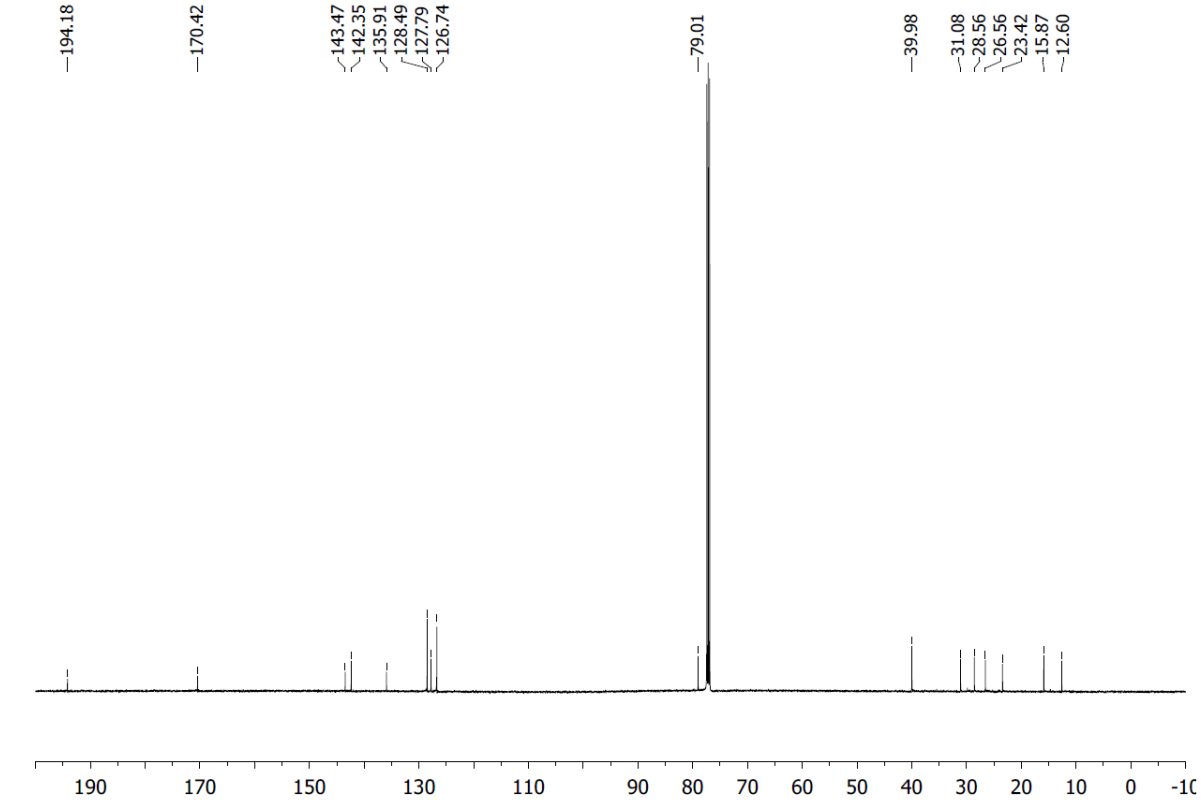
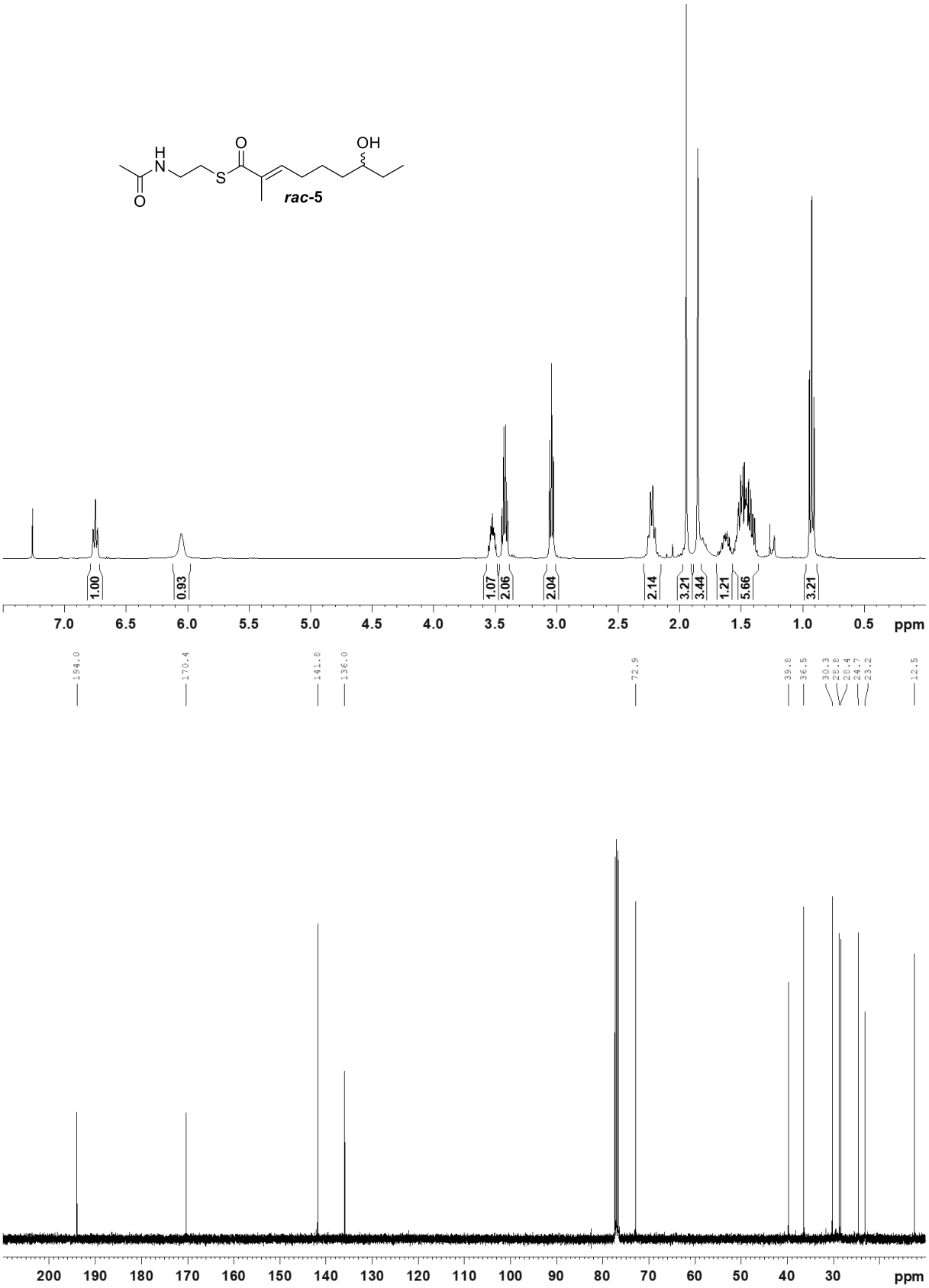


Figure S41. ^{1}H NMR analysis of the 9-AMA ester of the ***rac*-(5)** fraction from the enzymatic conversion experiment ($CDCl_3$, 500 MHz). The conversion was determined from the integrals corresponding to 7-H at (δ=6.7 and 6.1 ppm). Besides the diastereomers, the spectrum also shows signals corresponding to dichloromethane as well as aromatic and aliphatic contaminations.

NMR spectra of starting materials






rac-5
1.00
0.93
1.07
2.06
2.04
2.14
3.21
3.44
1.21
5.66
3.21
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
194.0
170.4
141.8
136.0
72.9
39.8
36.5
30.3
28.8
28.4
24.7
23.2
12.5
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

References Supporting Information

[1] T. Hollmann, G. Berkhan, L. Wagner, K. H. Sung, S. Kolb, H. Geise, F. Hahn, *ACS Catal.* **2020**, *10*, 4973–4982.
[2] G. Berkhan, F. Hahn, *Angew. Chem. Int. Ed.* **2014**, *53*, 14240–14244.
[3] M. Frese, N. Sewald, *Angew. Chem. Int. Ed.* **2015**, *54*, 298–301.
[4] R. A. Sheldon, S. van Pelt, *Chem Soc Rev* **2013**, *42*, 6223–6235.
[5] W. D. Fiers, G. J. Dodge, Y. Li, J. L. Smith, R. A. Fecik, C. C. Aldrich, *Chem. Sci.* **2015**, *6*, 5027–5033.
[6] D. S. Brown, M. Bruno, R. J. Davenport, S. V. Ley, *Tetrahedron* **1989**, *45*, 4293–4308.
[7] F. Godin, M. Prévost, F. Viens, P. Mochirian, J.-F. Brazeau, S. I. Gorelsky, Y. Guindon, *J. Org. Chem.* **2013**, *78*, 6075–6103.
[8] M. Gutiérrez, A. R. Pereira, H. M. Debonsi, A. Ligresti, V. Di Marzo, W. H. Gerwick, *J. Nat. Prod.* **2011**, *74*, 2313–2317.