Heterogeneous & Homogeneous & Bio-
CHEMCATCHEM
CATALYSIS

Supporting Information

Development of an *R*-Selective Amine Oxidase with Broad Substrate Specificity and High Enantioselectivity

Rachel S. Heath, Marta Pontini, Beatrice Bechi, and Nicholas J. Turner*[a]

cctc_201301008_sm_miscellaneous_information.pdf

Contents

1. General chemicals and methods

1.1 Chemicals

Potassium monophosphate, dipotassium phosphate, sodium chloride, imidazole, lysozyme, , 4-aminoantipyrine and 2,4,6-Tribromo-3-hydroxybenzoic acid were from Sigma or Fisher.

(*S*)-Nicotine, 2-Isobutylazepane, and Anabasine were purchased from Sigma Aldrich, 2-Phenyl pyrrolidine from Apollo Scientific, 6,7-Dimethoxy-1-methyl-3,4-tetrahydroisoquinoline, 6,7-Dimethoxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline and 6,7-Diethoxy-1,2,3,4-tetrahydroisoquinoline were purchased from Acros Organics, while Nornicotine was from Alfa Aesar. 1-(Naphthalen-1-yl)-1,2,3,4-tetrahydroisoquinoline and 1-(2-bromophenyl)-1,2,3,4-tetrahydroisoquinoline were kindly donated by J. M. Rosello and Professor J. Clayden, Department of Chemistry, University of Manchester.

1-Phenyltetrahydroisoquinoline was obtained by reduction of the corresponding lactam with $NaBH_4/I_2$ instead of $LiAlH_4$ according to Amat *et al*.[1] N-Methyl-2-phenylpyrrolidine and 4-(Methylamino)-1-(3'-pyridyl)-1-butanone dihydrochloride (pseudoxynicotine) were prepared according to Dunsmore *et al*.[2] 6-Amino-nicotine and 6-Hydroxy nicotine were synthetized according to procedures published by Tachitschibabin and Kirssanow;[3] Crispine A was synthesized as in Rowles *et al*.[4] Methyl anabasine was obtained by methylation of anabasine with methyl iodide/sodium hydride.

Solvents were analytical or HPLC grade or were purchased and dried over molecular sieves where necessary.

1.2 Analysis methods

Column chromatography was performed on silica gel (Sigma-Aldrich, 220-440 mesh).

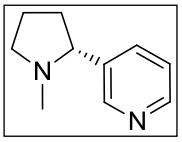
^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400.1 MHz or 399.9 MHz for ^{1}H and 100.6 MHz for ^{13}C) without additional internal standard. Chemical shifts are reported in δ values (ppm) and are calibrated against residual solvent signal. The following abbreviations were used to define the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad.

HPLC analysis was performed on an Agilent system equipped with a G1379A degasser, G1312A binary pump, a G1329 autosampler unit, a G1315B diode array detector and a G1316A temperature controlled column compartment. The columns used were CHIRALPAK® IC (5 µm particle size, 4.6 mm diameter x 250 mm), CHIRALPAK® IE (5 µm particle size, 4.6 mm diameter x 250 mm), CHIRALPAK® IA (5 µm particle size, 4.6 mm diameter x 250 mm) and CHIRALCEL® DAICEL OD-H (5 µm particle size, 4.6 mm diameter x 250 mm); conditions are indicated separately for each compound. GC analysis was performed on Agilent 6850 GCs equipped with a Gerstel Multipurposesampler MPS2L; Measurements were carried out using Varian Chirasil-Dex CB column 25 m × 0.25 mm × 0.26 µm film thickness or Supelco β-DEX 325 column, 30 m × 0.25 mm × 0.25 µm film thickness; conditions are indicated separately for each compound.

Optical rotation was measured at 28 °C on a Perkin–Elmer Polarimeter 341 against the sodium D-line.

2. Synthesis of substrates

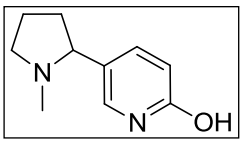
2.1 (*R*)-Nicotine (4)



(*S*)-nicotine (500 mg, 3 mmol) and BH_3NH_3 (360 mg, 12 mmol) were dissolved in potassium phosphate buffer (30 mL, 1M, pH = 7.8). Cell pellet (5 g) from *E. coli* cultures containing MAO-N D5 was added to the solution. The bottle was placed in a shaking incubator and shaken at 37 °C and 250 rpm. When HPLC analysis (CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*S*)] = 18.7 min, *Rt* [(*R*)] = 20.5 min) showed the conversion reached > 98 % and the amount of imine < 2% aqueous, aqueous NaOH (2 mL, 10 M) and CH_2Cl_2 (200 mL) were added. The mixture was transferred to Falcon tubes and layers were separated by centrifugation (4000 rpm, 5 min) and the aqueous phase was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic phases were dried with $MgSO_4$ and concentrated under reduced pressure to give the product (*R*)-nicotine (429 mg, 86% yield) as a yellow liquid.

$[\alpha]_D^{25}$ = + 133.0 (*c* = 1.0, $CHCl_3$). (lit.[5] (*S*)-nicotine $[\alpha]_D^{20}$ = -138.0 (c= 1, $CHCl_3$)).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (brd, *J*=2.1 Hz, 1H), 8.50 (dd, *J*=4.7 Hz, 1.7 Hz, 1H), 7.72 (dt, *J*=7.8, 1.9 Hz, 1H), 7.26 (app q, *J*=5.1 Hz, 1H), 3.26 (t, *J*=8.0 Hz, 1H), 3.10 (t, *J*=8.3 Hz, 1H), 2.33 (app q, *J*=8.0 Hz, 1H), 2.26 – 2.19 (m, 1H), 2.18 (s, 3H), 2.06 – 1.93 (m, 1H), 1.88 – 1.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.8, 148.9, 138.9, 135.1, 123.8, 69.1, 57.2, 40.5, 35.3, 22.8.

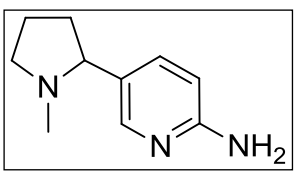
2.2 6-Hydroxy-nicotine (1)



Under nitrogen atmosphere, to a solution of 6-amino-nicotine·HCl (2.5 g, 11.7 mmol) in sulphuric acid (5% in water, 20 mL), a solution of sodium nitrite (8.3 g, 120 mmol) was added and the mixture was stirred for an additional hour. The reaction was neutralised with aqueous NaOH (2 mL, 5 M) and then extracted with CH_2Cl_2 (3 x 80 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH 9:1) gave the product 6-hydroxy nicotine (500 mg, 24% yield) as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.34 (bs, 1H), 7.53 (dd, *J*=9.4, 2.1 Hz, 1H), 7.25 (s, 1H), 6.56 (d, *J*=9.4 Hz, 1H), 3.20 – 3.12 (m, 1H), 2.82 (t, *J*=8.3 Hz, 1H), 2.28 – 2.15 (m, 1H), 2.11 (s, 3H), 2.10 – 2.00 (m, 1H), 1.98 – 1.82 (m, 1H), 1.82 – 1.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 141.5, 132.8, 121.6, 120.4, 67.6, 56.6, 40.0, 33.7, 22.3. TOF-Ms (*m/z*) = 179.2 $[M+H]^+$.

2.3 6-Amino-nicotine (8)

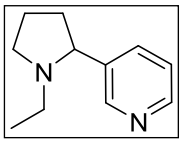


Under nitrogen atmosphere, to a solution of (*S*)-nicotine (25 g, 154 mmol) in xylene (50 mL), sodium amide (12 g, 307 mmol) was added portionwise and the mixture was stirred and heated under

reflux. After 5 hours, the reaction mixture was cooled to rt and poured onto ice; concentrated hydrochloric acid was then added unti a pH of 2 was reached and the two phases separated. The aqueous phase was neutralised with 1 M NaOH and then extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH 95:5) afforded the 6-amino-nicotine (19.4 g, 71% yield) as a brown oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J*=2.1 Hz, 1H), 7.41 (dd, *J*=8.5, 2.3 Hz, 1H), 6.45 (t, *J*=8.4 Hz, 1H), 4.53 (bs, 2H), 3.19 – 3.13 (m, 1H), 2.89-2.82 (m, 1H), 2.24 – 2.14 (m, 1H), 2.09 (s, 3H), 2.09 – 2.00 (m, 1H), 1.95 – 1.81 (m, 1H), 1.79-1.59 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.0, 147.5, 136.9, 127.9, 108.9, 68.6, 56.9, 40.2, 34.5, 22.3. TOF-MS (*m/z*) = 178.1 $[M+H]^+$.

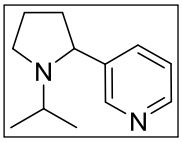
2.4 N-Ethyl nornicotine (9)



Nornicotine (105 mg, 0.71 mmol) was dissolved in DMF (4 mL) and NaH (0.71 mmol) 60 % dispersion in oil was added. Ethyl iodide (0.85 mmol) was added and the reaction was stirred at rt for 3 hours. TLC of the reaction mixture revealed that the starting material was still present, consequently ethyl iodide (0.85 mmol) was added and the reaction temperature was increased to 60 °C for 2 hours. The reaction was quenched by the addition of water (2 mL) and the mixture extracted with toluene (3 x 5 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate/MeOH 9:1 + 1% Et_3N), to give the product N-Ethyl nornicotine (75 mg, 60 % yield) as yellow oil.

^{1}H NMR, ^{13}C NMR and Mass analysis for N-Ethyl nornicotine were consistent with those reported in the literature.[6]

2.5 N-Isopropyl nornicotine (10)

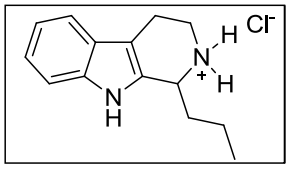


Nornicotine (100 mg, 0.675 mmol) was dissolved in acetone (3 mL) and a catalytic amount of acetic acid (0.050 mL) was added. The reaction was stirred at rt for 1 hour. Sodium triacetoxyborohydride (0.945 mmol) was added and the reaction was stirred at room temperature for an additional 2 hours. The reaction was then quenched by the addition of water (2 mL) and aqueous NaOH 50 % w/w (2 mL), and extracted with CH_2Cl_2 (2 mL), and the organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate/MeOH 9:1, 1% Et_3N), to give the product N-Isopropyl nornicotine (90 mg, 70 % yield) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) *δ* 8.54 (d, *J*=2.0 Hz, 1H), 8.44 (dd, *J*=4.7 and 1.7 Hz, 1H), 7.71 (d, *J*=7.7 Hz, 1H), 7.19 – 7.22 (m, 1H), 3.67 (t, *J*=7.6 Hz, 1H), 3.06 – 3.11 (m, 1H), 2.69 –2.76 (m, 1H), 2.55 – 2.63 (m, 1H), 2.10 – 2.18 (m, 1H), 1.81 – 1.90 (m, 1H), 1.70 – 1.79 (m, 1H), 1.57 – 1.66 (m, 1H), 0.98 (d, *J*=6.7 Hz, 3H), 0.90 (d, *J*=6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) *δ*

149.3, 148.1, 141.2, 134.8, 123.4, 62.4, 49.0, 46.7, 35.7, 23.0, 22.4, 15.4. TOF-Ms (*m/z*) = 191.1 $[M+H]^+$.

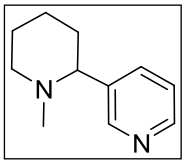
2.6 1-Propyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-2-ium (22)



Tryptamine hydrochloride (197 mg, 1 mmol) and butyraldehyde (90 µL, 1 mmol) were added to aqueous maleic acid buffer (10 mM, pH 2.0, total volume 2 mL) and the solution was stirred overnight at 60 °C. The salt precipitate and was filtered and washed with water (2 mL) to afford the product 1-Propyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-2-ium (187 mg, 75% yield).

^{1}H NMR (400 MHz, CD_3OD) δ 7.49 (d, *J*=7.8 Hz, 1H), 7.38 (d, *J*=8.1 Hz, 1H), 7.17 (t, *J*=7.6 Hz, 1H), 7.07 (t, *J*=7.4 Hz, 1H), 4.70 (dd, *J*=5.4, 3.5 Hz, 1H), 3.81 – 3.70 (m, 1H), 3.45 (ddd, *J*=12.6, 9.3, 5.8 Hz, 1H), 3.20 – 2.98 (m, 2H), 2.34 – 2.19 (m, 1H), 2.01 – 1.87 (m, 1H), 1.71 – 1.55 (m, 2H), 1.12 (t, *J*=7.3 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 138.3, 130.3, 127.4, 123.5, 120.6, 119.1, 112.3, 107.1, 54.9, 43.1, 35.4, 28.7 19.5, 14.2.

2.7 3-(1-Methylpiperidin-2-yl)pyridine (33)



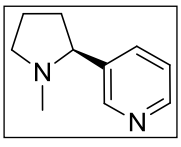
Anabasine (100 mg, 0.62 mmol) was dissolved in DMF (4 mL) and NaH (1.5 mg, 0.62 mmol, 60 % dispersion in oil) was added. Methyl iodide (0.74 mmol) was added and the reaction was stirred at rt for 3 hours. TLC of the reaction mixture revealed that the starting material was still present, consequently the reaction temperature was increased to 60 °C for 2 hours. The reaction was quenched by the addition of water (2 mL) and the mixture extracted with toluene (3 x 5 mL), and the combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate/MeOH 9:1 + 1% Et_3N), to give the product 3-(1-Methylpiperidin-2-yl)pyridine (65 mg, 60 % yield) as a yellow oil.

^{1}H and ^{13}C NMR data for 3-(1-methylpiperidin-2-yl)pyridine were consistent with those reported in the literature.[7]

3. Mechanism experiments

3.1 Study of the stereoinversion of (*R*)-nicotine (4) using wildtype 6-HDNO

3.1.1 Preparative procedure



In a 500 mL screw cap bottle, (*R*)-nicotine (100 mg, 0.62 mmol), BH_3NH_3 (74 mg, 2.47 mmol) and whole cells wildtype 6-HDNO (2 g) were dissolved in potassium phosphate buffer (30 mL, 1 M, pH = 7.8). The reaction was performed at 37 °C with shaking at 250 rpm. After 16 hours HPLC analysis (CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*S*)] = 18.7 min, *Rt* [(*R*)] = 20.5 min) showed full conversion to the (*S*)-enantiomer and the reaction was worked up by adding aqueous 10 M NaOH. The aqueous

phase was extracted with CH_2Cl_2 (3 × 30 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give (*S*)-nicotine (93 mg, 93% yield) as a yellow liquid.

$[\alpha]_D^{28}$ = - 129.0 (*c* = 1.0, $CHCl_3$). (lit. [5] $[\alpha]_D^{20}$ = -138.0 (c= 1, $CHCl_3$)).
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (b, *J*=2.1 Hz, 1H), 8.50 (dd, *J*=4.7, 1.7 Hz, 1H), 7.72 (dt, *J*=7.8, 1.9 Hz, 1H), 7.26 (app q, *J*=5.1, 1H), 3.26 (t, *J*=8.0 Hz, 1H), 3.10 (t, *J*=8.3 Hz, 1H), 2.33 (app q, *J*=8.0 Hz, 1H), 2.26 – 2.19 (m, 1H), 2.18 (s, 3H), 2.06 – 1.93 (m, 1H), 1.88 – 1.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.8, 148.9, 138.9, 135.1, 123.8, 69.1, 57.2, 40.5, 35.3, 22.8.

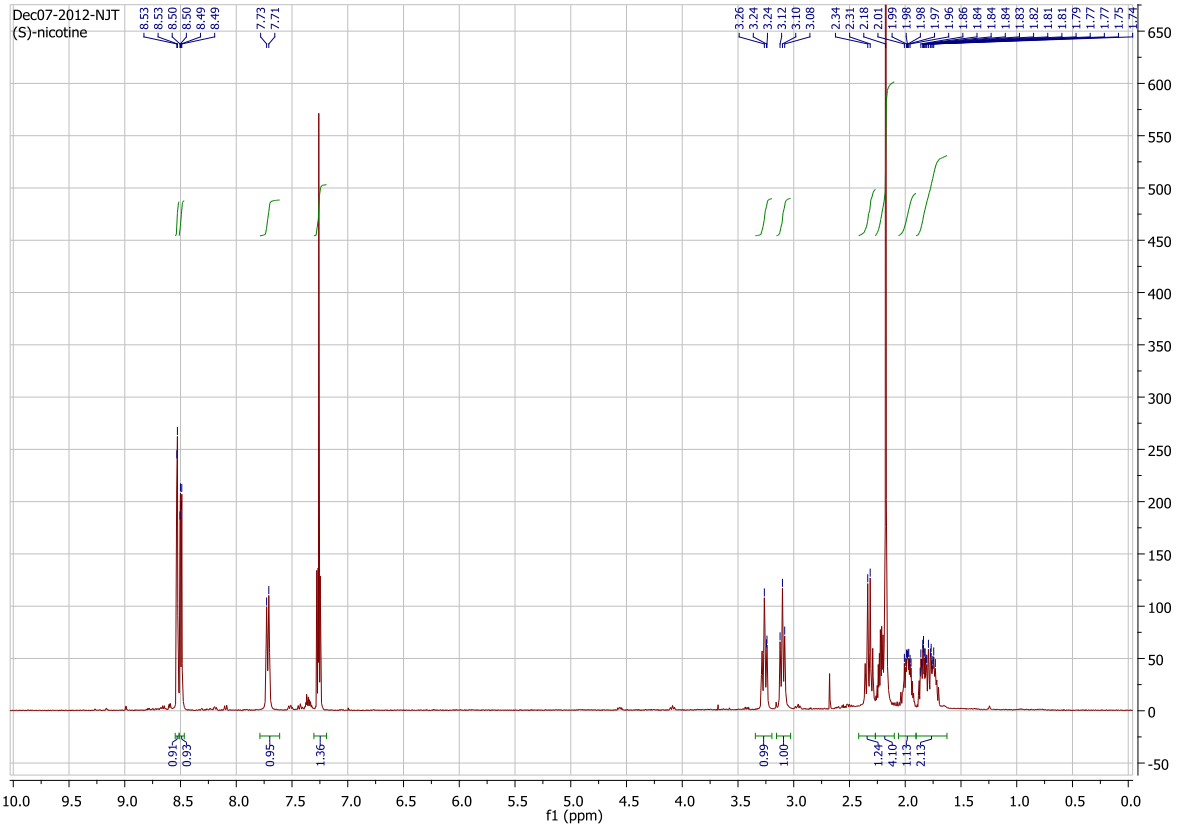


Figure S1. *^{1}H NMR (400 MHz, $CDCl_3$) of the crude extracts of the product (S)-nicotine from the biotransformation with wildtype 6-HDNO.*

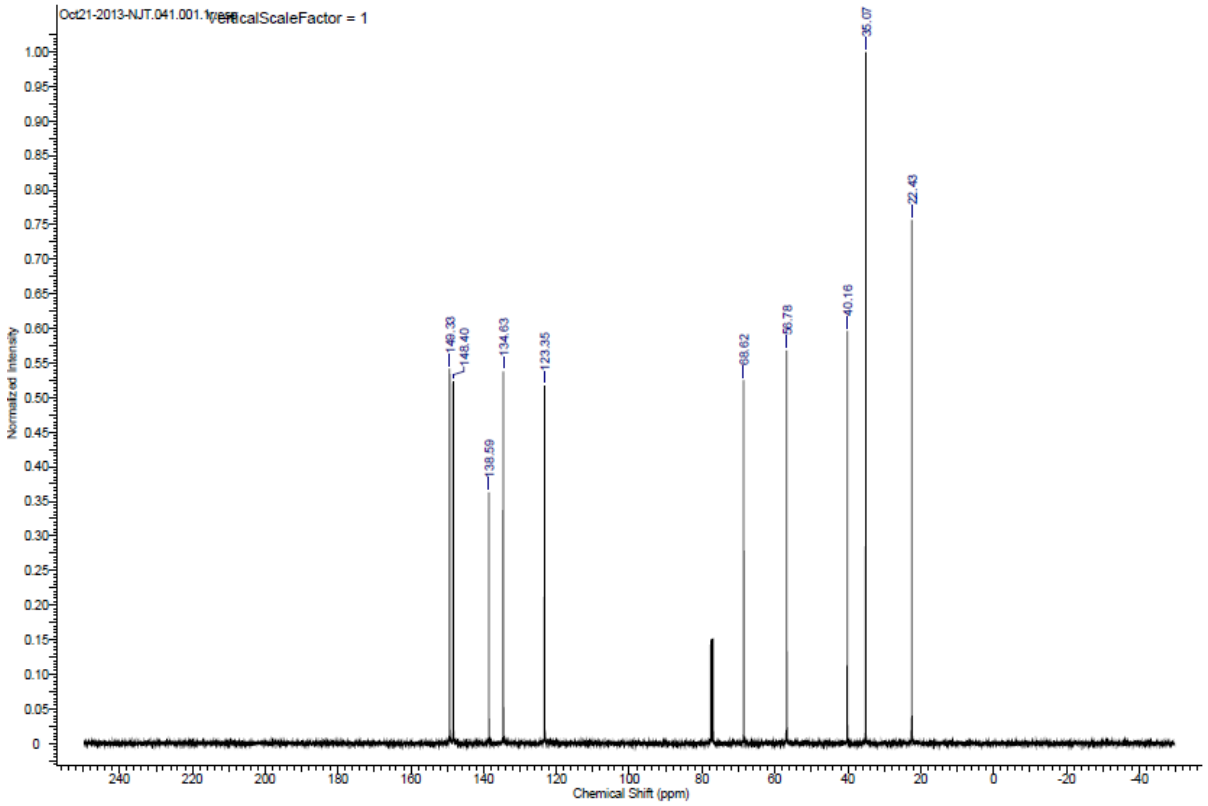
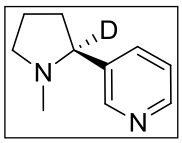


Figure S2. *^{13}C NMR (100 MHz, $CDCl_3$) of the crude extracts of the product (S)-nicotine from the biotransformation with wildtype 6-HDNO.*

3.1.2 Deuteration study (A): using a deuterated reducing agent



The reaction was set up using (*R*)-nicotine (50 mg, 0.31 mmol), whole cells wildtype 6-HDNO (1.5 g) in potassium phosphate buffer (30 mL, 1 M, pH = 7.8), performed at 37 °C with shaking at 250 rpm and monitored by HPLC (CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*S*)] = 18.7 min, *Rt* [(*R*)] = 20.5 min). After 16 hours the disappearance of the substrate was detected, together with the absence of formation of any other peaks in the HPLC profile (corresponding for example to pseudoxynicotine (open form of nicotine)). $NaBD_3CN$ (77.5 mg, 1.23 mmol) was added and the reaction mixture was left overnight at 37 °C, with shaking at 250 rpm. After observing the full conversion to the (*S*)-enantiomer by HPLC, adding aqueous NaOH (2 mL, 10 M) was added and the mixture extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was then continuously extracted overnight with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give (*S*)-nicotine (48 mg, 96% yield) and no presence of 4-(methylamino)-1-(3-pyridyl)-1-butanol as by-product).

^1H NMR (Figure S3, 400 MHz, $CDCl_3$) and ^{13}C NMR (Figure S4, 100 MHz, $CDCl_3$) differ from the corresponding NMR spectrums of nicotine: the signal at 3.10 ppm (for the ^1H spectrum) and at 69.1 ppm (for the ^{13}C NMR spectrum) corresponding to the CH_α are not present.

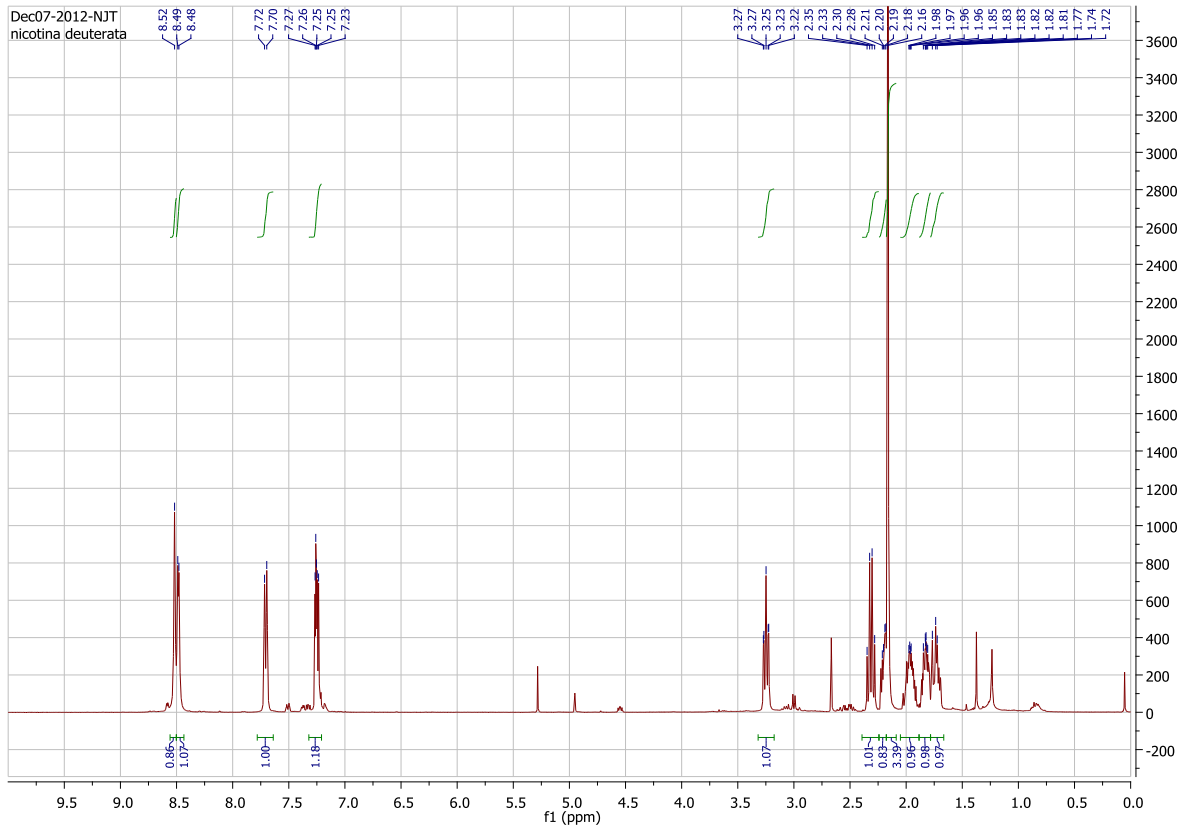


Figure S3. *^{1}H NMR (400 MHz, $CDCl_3$) of the crude extracts of the deuterated (S)-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$.*

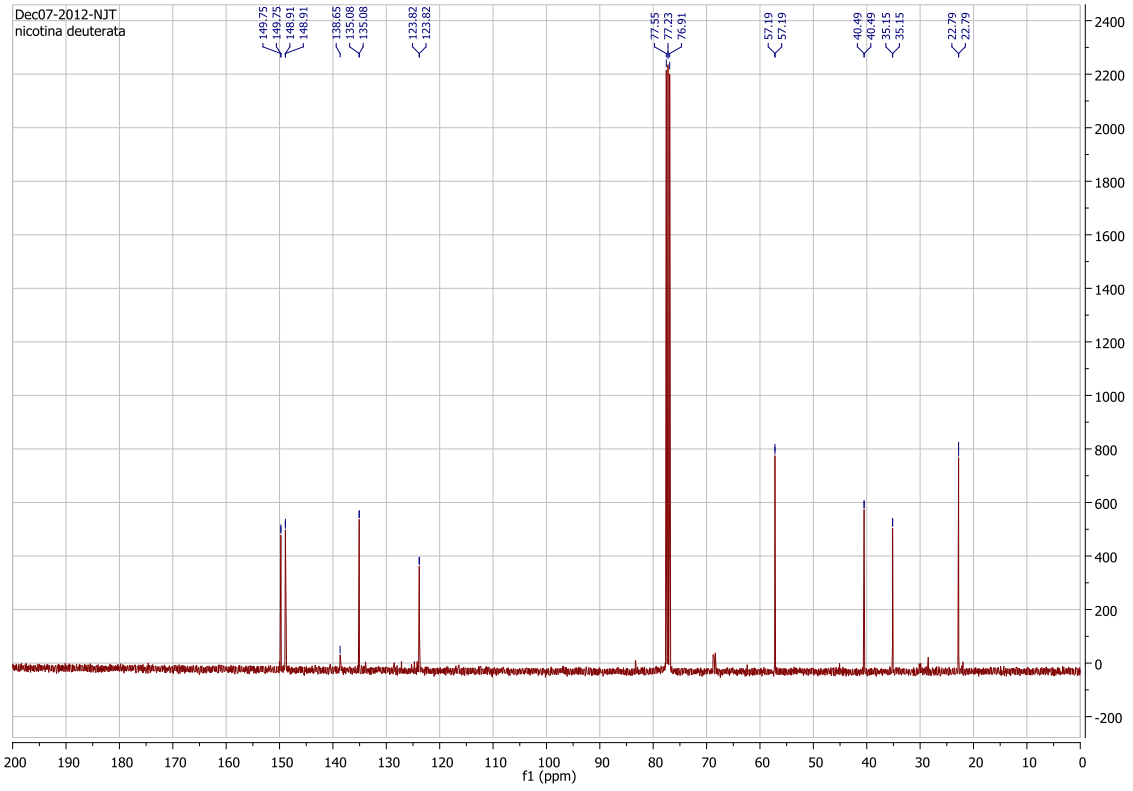
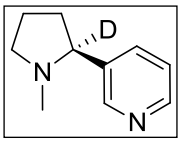


Figure S4. *^{13}C NMR (100 MHz, $CDCl_3$) of the crude extracts of the deuterated (S)-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$.*

3.1.3 Deuteration study (B): using a deuterated reducing agent and deuterium oxide



The reaction was set up using (*R*)-nicotine (50 mg, 0.31 mmol), freeze-dried whole cells wildtype 6-HDNO (1.5 g) and $NaBD_3CN$ (77.5 mg, 1.23 mmol) in D_2O-potassium phosphate buffer (30 mL, 1 M, pH = 7.8) and performed at 37 °C with shaking at 250 rpm. After 16 hours HPLC analysis (CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*S*)] = 18.7 min, *Rt* [(*R*)] = 20.5 min) showed full conversion to product. Aqueous NaOH (2 mL, 10 M) was added and the mixture extracted with CH_2Cl_2 (3 × 50 mL), then the aqueous phase was continuously extracted overnight with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to give (*S*)-nicotine (47 mg, 94% yield) as a yellow liquid. There was no presence of 4-(methylamino)-1-(3-pyridyl)-1-butanol as product or by-product.

^1H NMR (Figure S5, 400 MHz, $CDCl_3$) and ^{13}C NMR (Figure S6, 100 MHz, $CDCl_3$) spectra again differ from the corresponding NMR spectrumss of nicotine: the signal at 3.10 ppm (for the protonic spectrum) and at 69.1 ppm (for the ^{13}C NMR) corresponding to the CH_α are not present

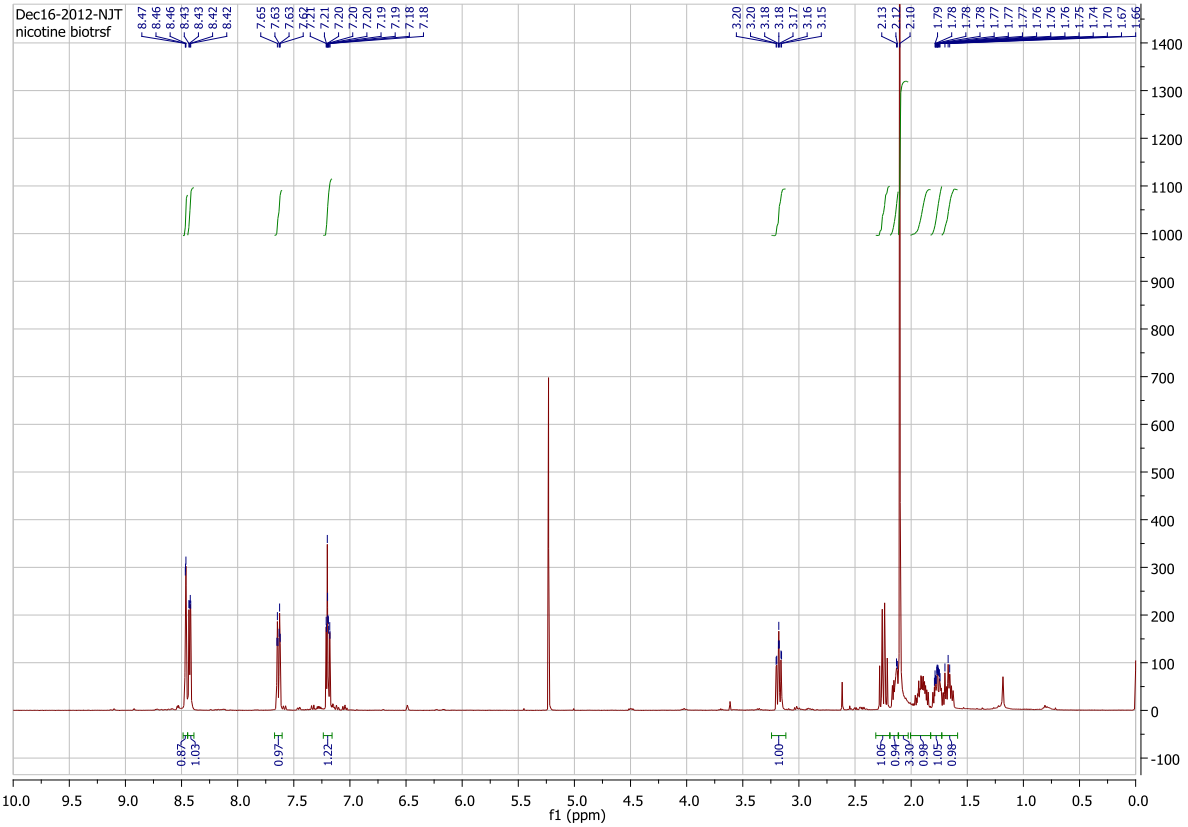


Figure S5. *^1H NMR (400 MHz, $CDCl_3$) of the crude extracts of the deuterated (S)-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$ in deuterium oxide .*

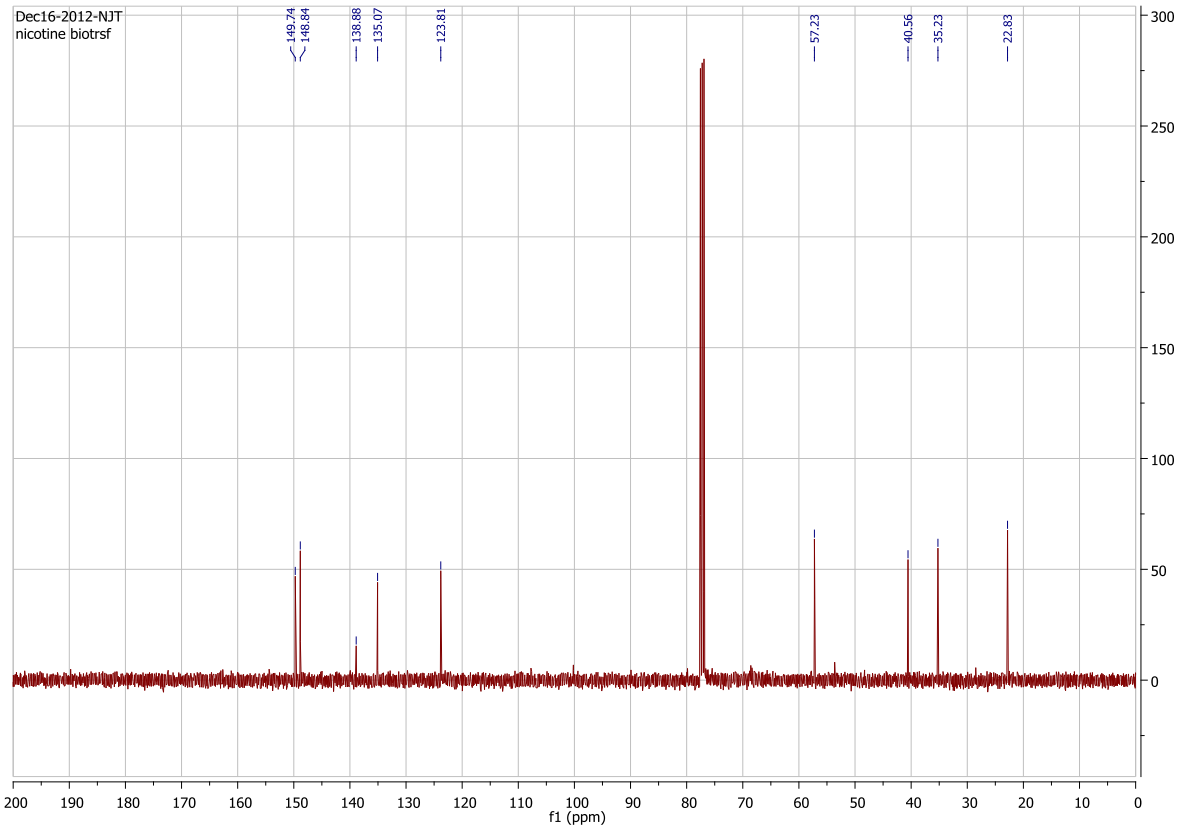


Figure S6. *^{13}C NMR (100 MHz, $CDCl_3$) of the crude extracts of the deuterated (S)-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$ in deuterium oxide.*

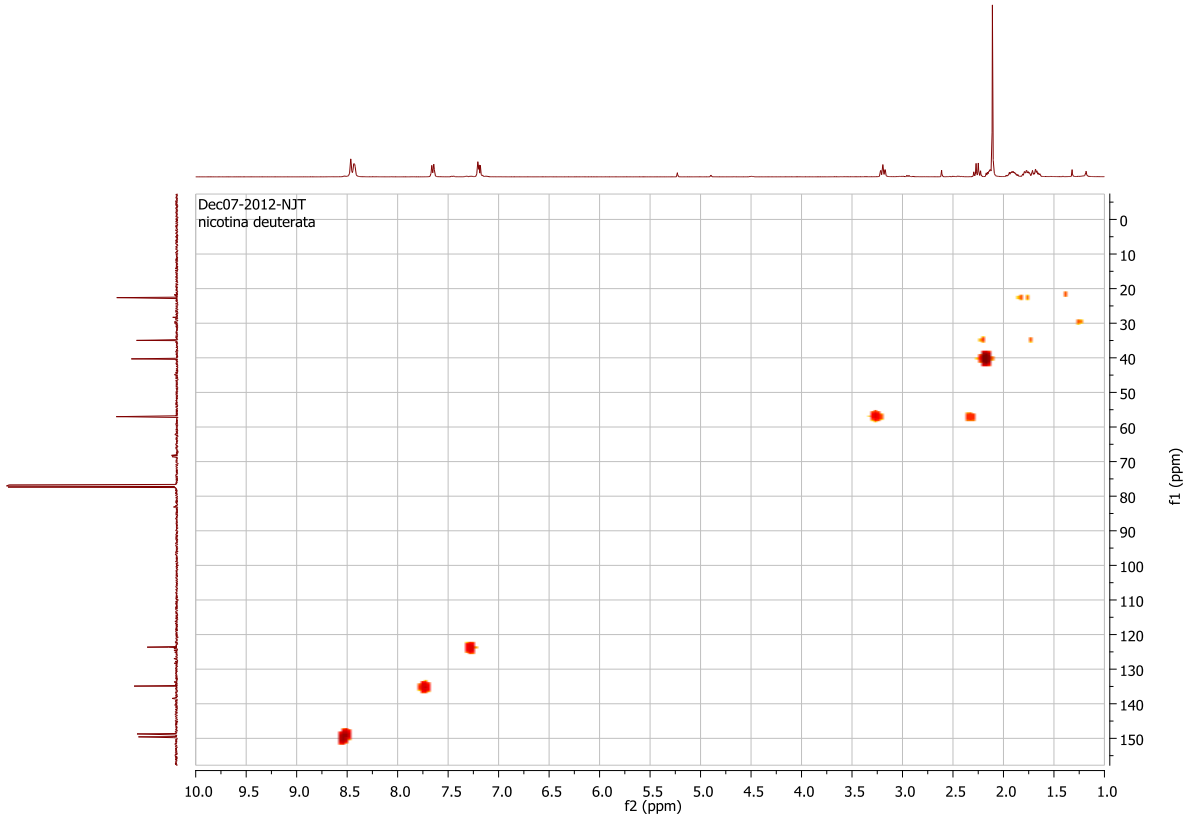
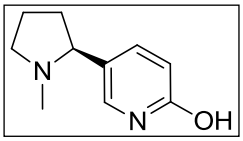


Figure S7. *HSQC spectrum of the crude extracts of the deuterated (S)-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$ in deuterium oxide.*

3.2 Study of the deracemisation of 6-hydroxy-nicotine (1) using wildtype 6-HDNO

3.2.1 Preparative procedure



In a 500 mL screw cap bottle, (*rac*)-6-hydroxy-nicotine (100 mg, 0.56 mmol), BH_3NH_3 (67 mg, 2.2 mmol) and purified wildtype 6-HDNO (10 mg) were dissolved in potassium phosphate buffer (30 mL, 1 M, pH = 7.8). The reaction was performed at 37 °C with shaking at 250 rpm. After 6 hours HPLC analysis (CHIRALCEL® DAICEL IA column; flowrate 1.5 mL / min; UV 280 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 19.8 min, *Rt* [(*S*)]) showed full conversion to the (*S*)-enantiomer. Aqueous NaOH (2 mL, 10 M) was added and the mixture extracted with CH_2Cl_2 (3 × 40 mL) and the combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure. The residue was, purified by column chromatographic on silica gel (CH_2Cl_2/MeOH 90:10) to give (*S*)-6-hydroxy-nicotine (55 mg, 55% yield) as a brown solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 13.34 (bs, 1H), 7.53 (dd, *J*=9.4, 2.1 Hz, 1H), 7.25 (s, 1H), 6.56 (d, *J*=9.4 Hz, 1H), 3.20 – 3.12 (m, 1H), 2.82 (t, *J*=8.3 Hz, 1H), 2.28 – 2.15 (m, 1H), 2.11 (s, 3H), 2.10 – 2.00 (m, 1H), 1.98 – 1.82 (m, 1H), 1.82 – 1.58 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 141.5, 132.8, 121.6, 120.4, 67.6, 56.6, 40.0, 33.7, 22.3.

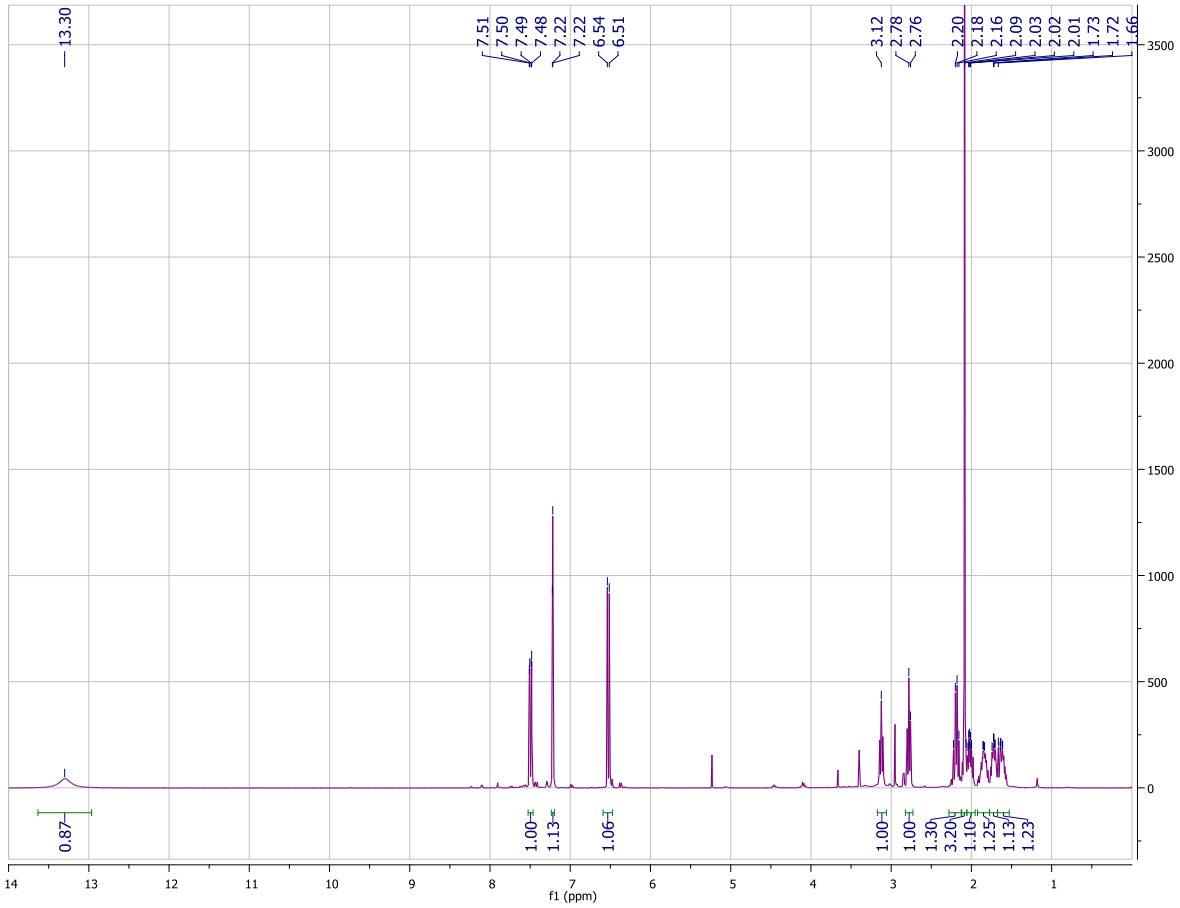


Figure S8. *^{1}H NMR (400 MHz, $CDCl_3$) of the crude extracts of the product (S)-6-hydroxy-nicotine from the biotransformation with wildtype 6-HDNO.*

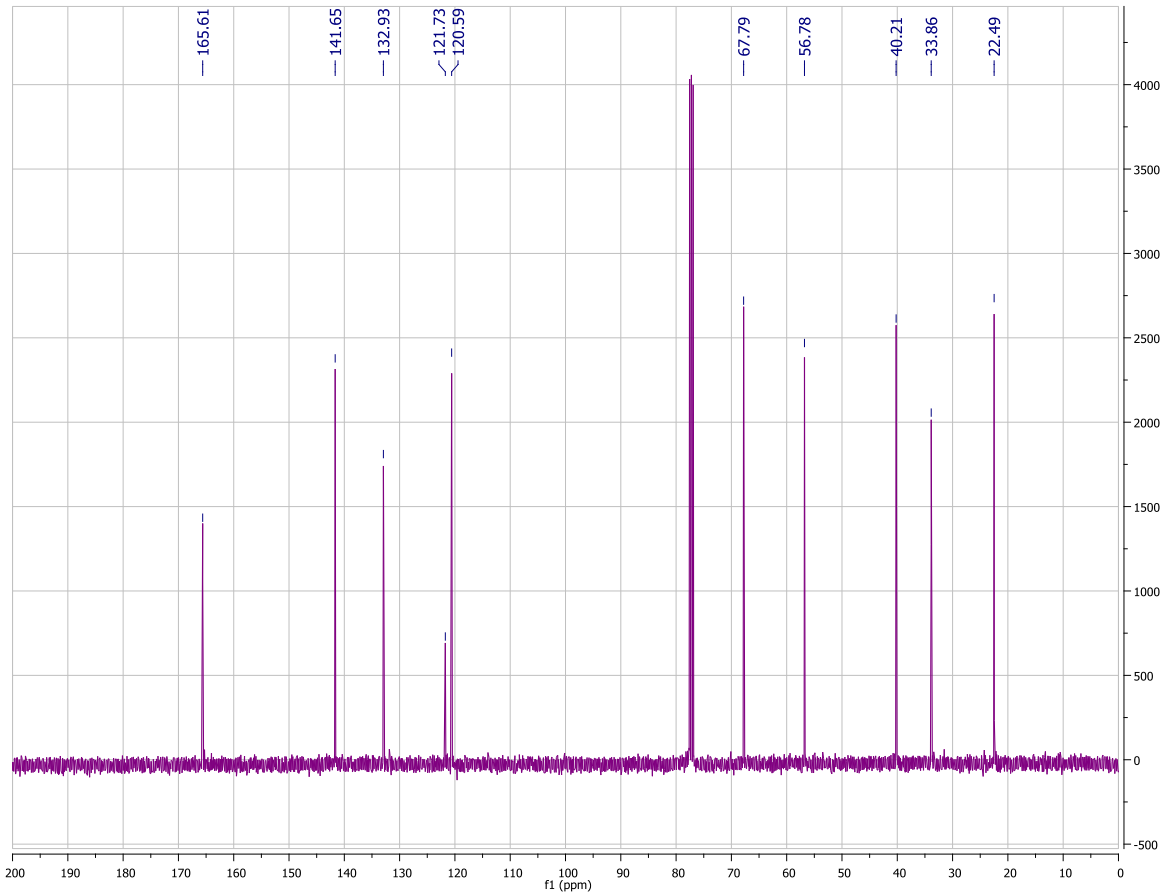
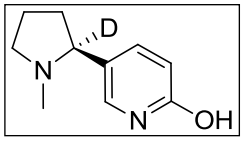


Figure S9. *^{13}C NMR (100 MHz, $CDCl_3$) of the product (S)-6-hydroxy-nicotine from the biotransformation with wildtype 6-HDNO.*

3.2.2 Deuteration study: using a deuterated reducing agent



The reaction was set up using (*rac*)-6-hydroxy-nicotine (50 mg, 0.28 mmol), $NaBD_3CN$ (73.8 mg, 1.12 mmol) and whole cells wildtype 6-HDNO (1.5 g) in potassium phosphate buffer (30 mL, 1 M, pH = 7.8). The reaction was performed at 37 °C with shaking at 250 rpm and monitored by HPLC (CHIRALCEL® DAICEL IA column; flowrate 1.5 mL/min; UV 280 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 19.8 min, *Rt* [(*S*)]). After 8 hours HPLC analysis showed full conversion, aqueous 10 M NaOH (2 mL, 10 M) was addedand the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The aqueous phase was then continuously extracted overnight with CH_2Cl_2. The combined organic phases were dried over $MgSO_4$, concentrated under reduced pressure and then purified on silica gel (CH_2Cl_2/MeOH 90:10) to give (*S*)-6-hydroxy-nicotine (38 mg, 76% yield) as a brown solid.

^1H NMR spectrum (Figure S10, 400 MHz, $CDCl_3$) differs for one signal's integration from the ^1H NMR spectrum of racemic 6-hydroxy-nicotine. The substrate of this biotransformation is the racemic 6-hydroxy-nicotine and, consequently, the product will have the deuterium in the α position with ratio CHα: CDα = 1:1.

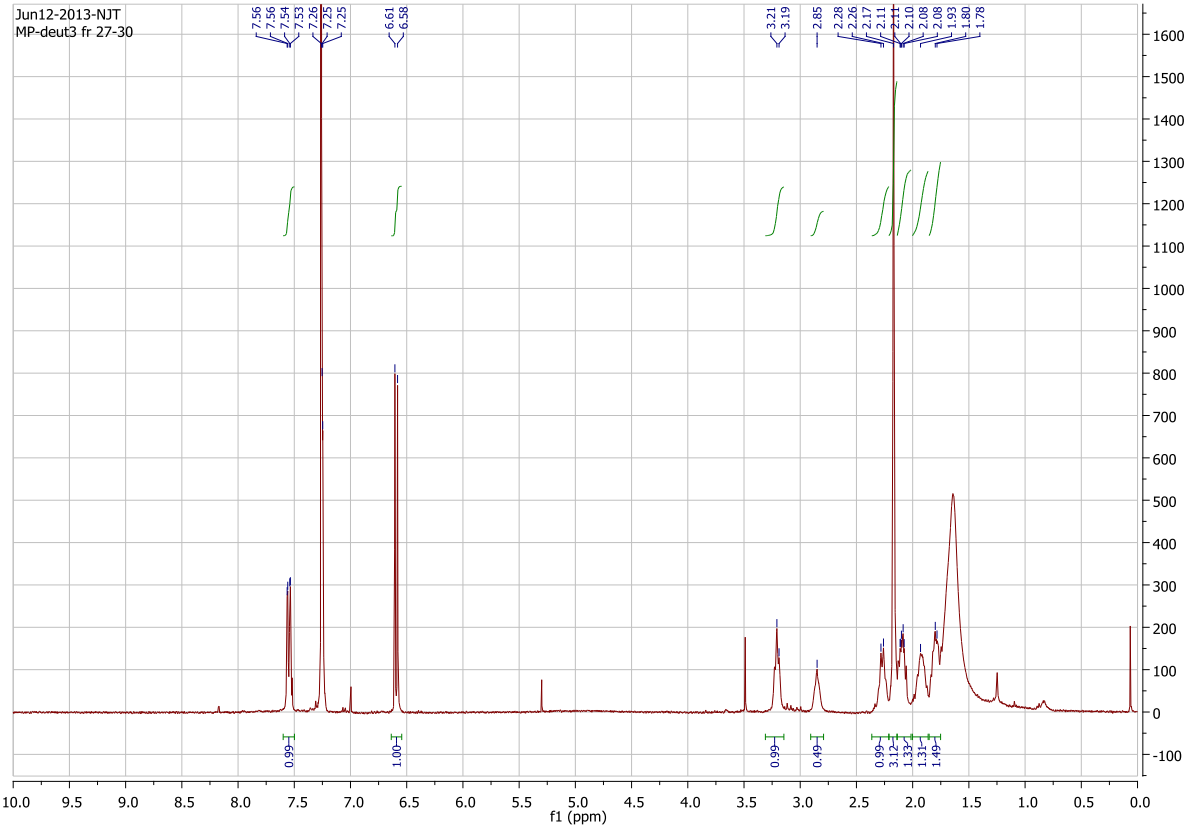


Figure S10. *^{1}H NMR (400 MHz, $CDCl_3$) of the product (S)-6-hydroxy-nicotine from the biotransformation with wildtype 6-HDNO and $NaBD_3CN$.*

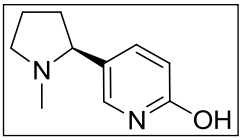
4. Biotransformations – procedures and analyses

4.1 General procedure for biotransformations

Typically, half a gram of *E. coli* cells containing the expressed HDNO protein were resuspended in 1 M phosphate buffer (pH = 7.8) to a final volume of 5 mL. Substrates were added for a final concentration of 10 mM, followed by 40 mM (final concentration) of NH_3BH_3. The biotransformation was performed at 37 °C with shaking at 250 rpm. Samples (300 µl) were basified with 10 M NaOH and extracted with 1 mL dichloromethane of which typically 10 µL was injected onto the column for analysis by HPLC or GC methods. In the analysis details below, this general procedure was followed to prepare the samples unless otherwise stated. For samples analysed by OD-H column methods, dichloromethane was evaporated and the product redissolved in *tert*-butyl methyl ether.

4.2 Analysis of biotransformations carried out with wildtype 6-HDNO

4.2.1 (S)-6-Hydroxy nicotine (1)



The biotransformation was carried out with (*rac*)-(1). After 1 hour HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® IA column; flowrate 1.5 mL / min; UV 280 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA, *Rt* [(*R*)] = 19.8 min, *Rt* [(*S*)] = 23.1 min.

The absolute stereochemistry was assigned by previous reports in which the enzyme is only active on the (*R*)-enantiomer and not the (*S*)-enantiomer,[8,9] and by comparison to nicotine.

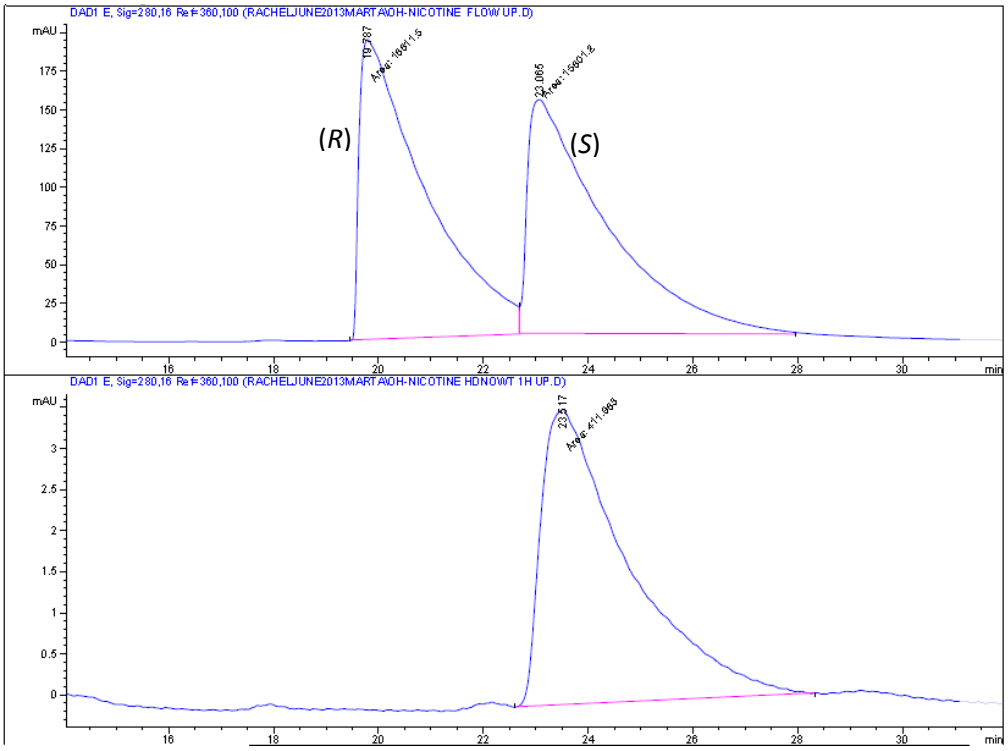
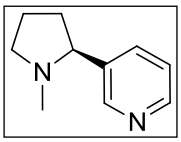


Figure S11. *Chiral phase HPLC trace of* (*rac*)-(*1*) *conversion with 6-HDNO showing the signals of* (*rac*) *compound* (*1*) (*top*) *and the deracemization product* (S)-(*1*) (*bottom*).

4.2.2 (S)-Nicotine (4)



The biotransformation was carried out with (*R*)-(4) (made by deracemisation of (*S*)-nicotine with MAO-N, see 2.1.). After 16 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*S*)] = 18.7 min, *Rt* [(*R*)] = 20.5 min. The absolute configuration was assigned by comparison to a commercial standard of (*S*)-nicotine and by comparison to the deracemisation product with MAO-N ((*R*)-enantiomer).[10]

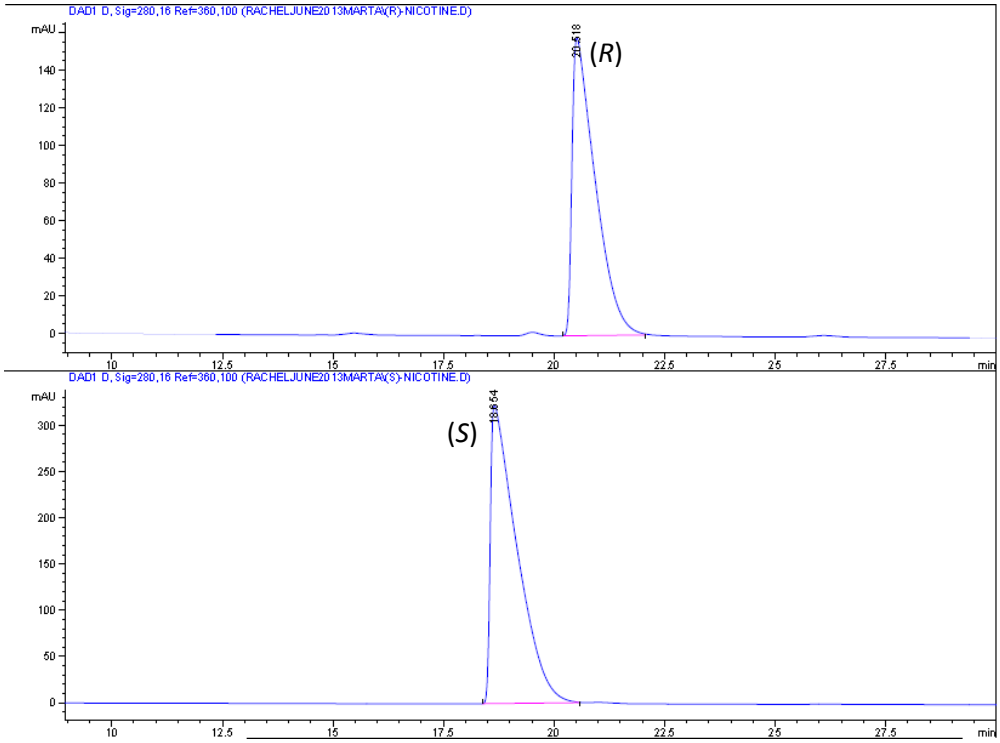
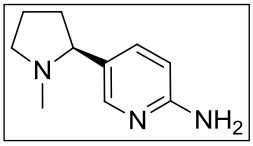


Figure S12. *Chiral phase HPLC trace of* (*R*)-(*4*) *conversion with 6-HDNO showing the signals of* (*R*)-(*4*) *substrate* (*top*) *and the deracemization product* (*S*)-(*4*) (*bottom*).

4.2.3 (S)-6-Amino nicotine (8)



The biotransformation was carried out with (*rac*)-(8). After 4 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 98:2 + 0.1% DEA; *Rt* [(*S*)] = 86.2 min, *Rt* [(*R*)] = 91.9 min. The absolute stereochemistry was assigned by analogy to 6-hydroxy nicotine and comparison to MAO-N (unpublished data).

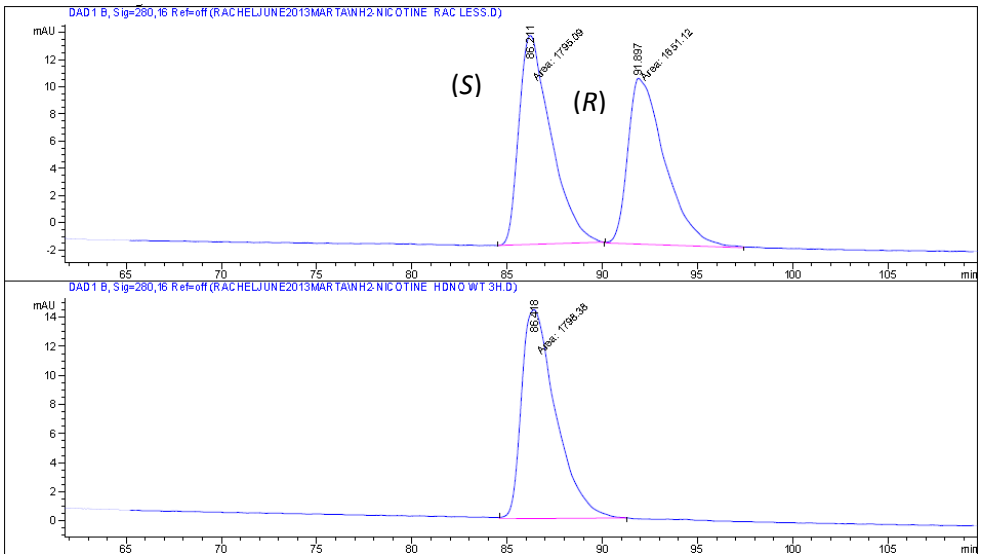
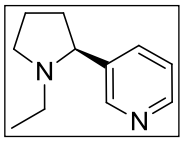


Figure S13. *Chiral phase HPLC trace of* (*rac*)-(*8*) *conversion with 6-HDNO showing the signals of racemic compound* (*8*) (*top*) *and the deracemization product* (S)-(*8*) (*bottom*).

4.2.4 (S)-N-Ethyl nornicotine (9)



The biotransformation was carried out with (*rac*)-(9). After 93 hours HPLC analysis showed 90 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 265 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 12.8 min, *Rt* [(*S*)] = 12.1 min. The absolute stereochemistry was assigned by analogy to nicotine.

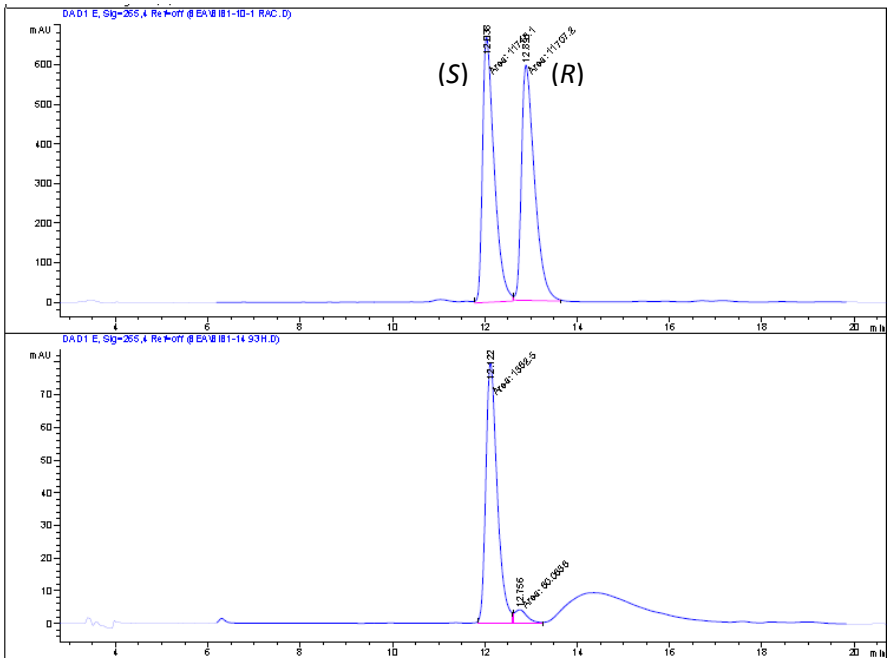
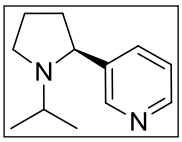


Figure S14. *Chiral phase HPLC trace of* (*rac*)-(*9*) *conversion with 6-HDNO showing the signals of racemic compound* (*9*) (*top*) *and the deracemization product* (S)-(*9*) (*bottom*).

4.2.5 (S)-N-Isopropyl nornicotine (10)



The biotransformation was carried out with (*rac*)-(10). After 96 hours HPLC analysis showed 54 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 265 nM; eluent = hexane/iPrOH 99:1 + 0.1% DEA; *Rt* [(*R*)] = 25.1 min, *Rt* [(*S*)] = 23.6 min. The absolute stereochemistry was assigned by analogy to nicotine.

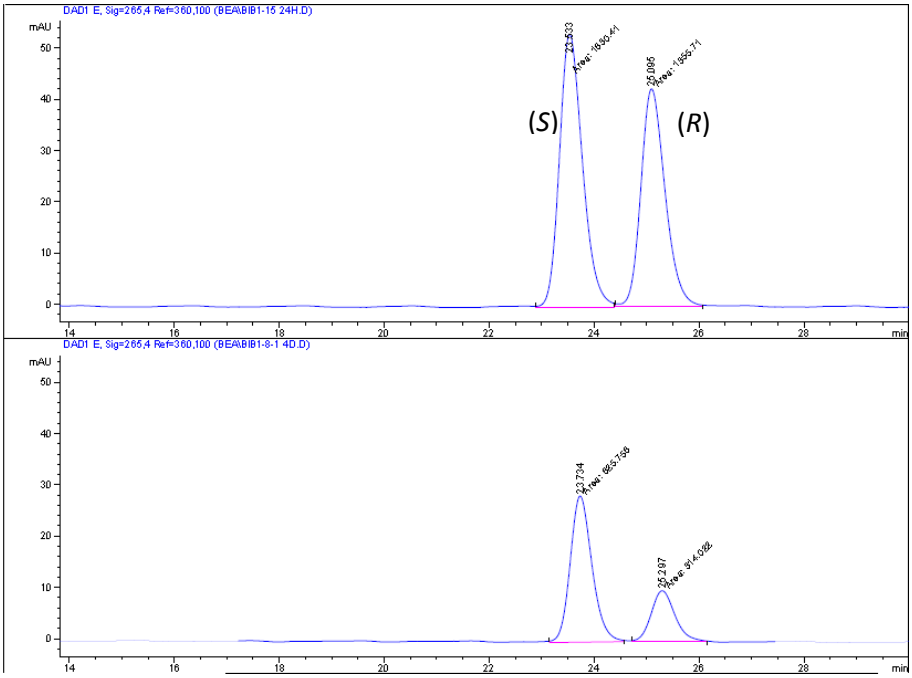
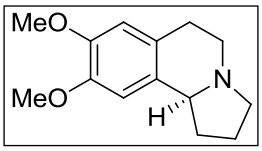


Figure S15. *Chiral phase HPLC trace of* (*rac*)-(*10*) *conversion with 6-HDNO showing the signals of racemic compound* (*10*) (*top*) *and the deracemization product* (S)-(*10*) (*bottom*).

4.2.6 (S)-Crispine A (26)



The biotransformation was carried out with (*rac*)-(26). After 4 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 254 nM; eluent= hexane/iPrOH 90:10 + 0.1% DEA; *Rt* [(*R*)] = 15.8 min, *Rt* [(*S*)] = 10.3 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer).[4]

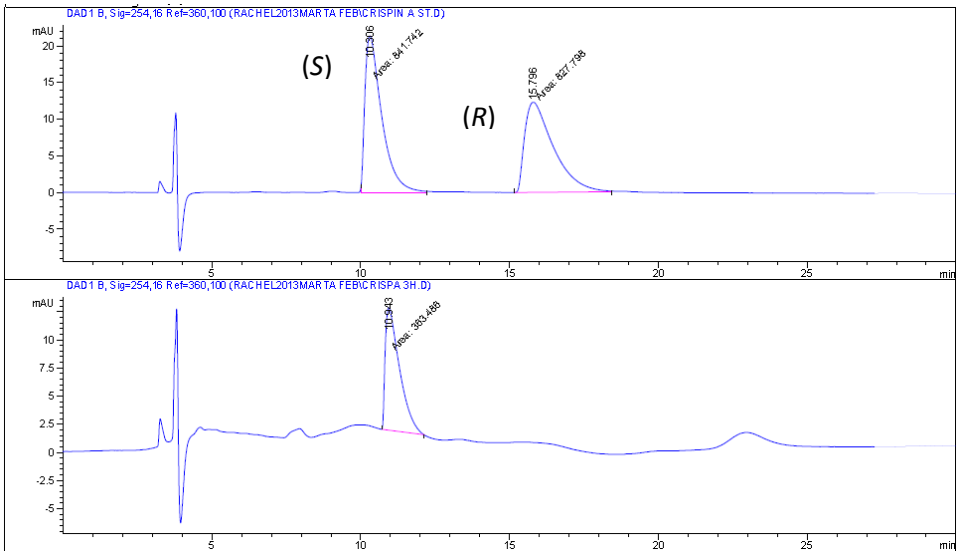
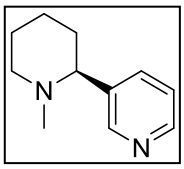


Figure S16. *Chiral phase HPLC trace of* (*rac*)-(*26*) *conversion with 6-HDNO showing the signals of racemic compound* (*26*) (*top*) *and the deracemization product* (S)-(*26*) (*bottom*).

4.2.7 (S)- 3-(1-Methylpiperidin-2-yl)pyridine (33)



The biotransformation was carried out with (*rac*)-(33). After 48 hours HPLC analysis showed 84 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL/min; UV 254 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*S*)] = 6.5 min, *Rt* [(*R*)] = 8.1 min. The absolute stereochemistry was assigned by comparison of its optical rotation with the literature (see preparative procedure).

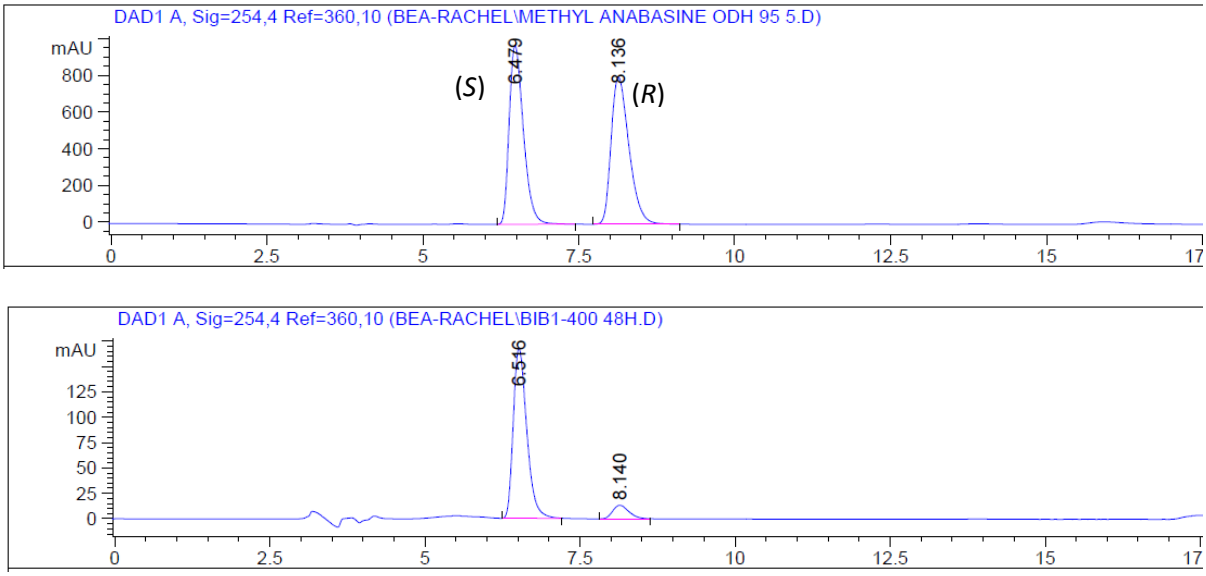
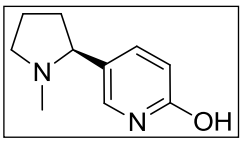


Figure S17. *Chiral phase HPLC trace of* (*rac*)-(*33*) *conversion with 6-HDNO showing the signals of racemic compound* (*33*) (*top*) *and the deracemisation product* (S)-(*33*) (*bottom*).

4.3 Analysis of biotransformations carried out with variant E350L/E352D

4.3.1 (S)-6-Hydroxy nicotine (1)



The biotransformation was carried out with (*rac*)-(1). After 1 hour HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL IA column; flowrate 1.5 mL / min; UV 280 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 19.8 min, *Rt* [(*S*)] = 23.1 min. The absolute stereochemistry was assigned by previous reports in which the enzyme is only active on the (*R*)-enantiomer and not the (*S*)-enantiomer.[8,9]

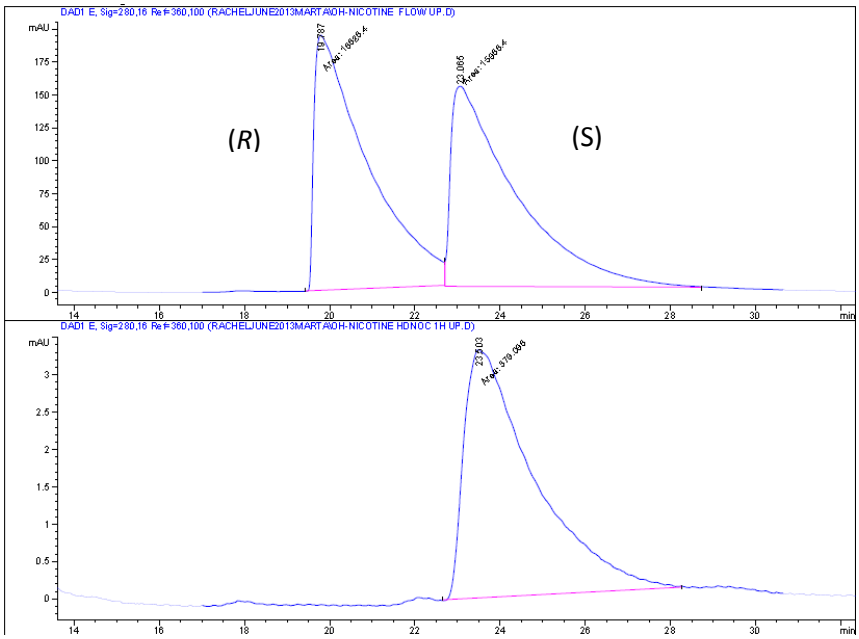
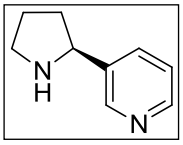


Figure S18. *Chiral phase HPLC trace of* (*rac*)-(*1*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*1*) (*top*) *and the deracemisation product* (S)-(*1*) (*bottom*).

4.3.2 (S)-Nornicotine (7)



The biotransformation was carried out with (*rac*)-(7). After 28 hours HPLC analysis showed 95 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 254 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 26.9 min, *Rt* [(*S*)] = 31.1 min.

The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer) (unpublished data) and analogy with 2-phenyl pyrrolidine.

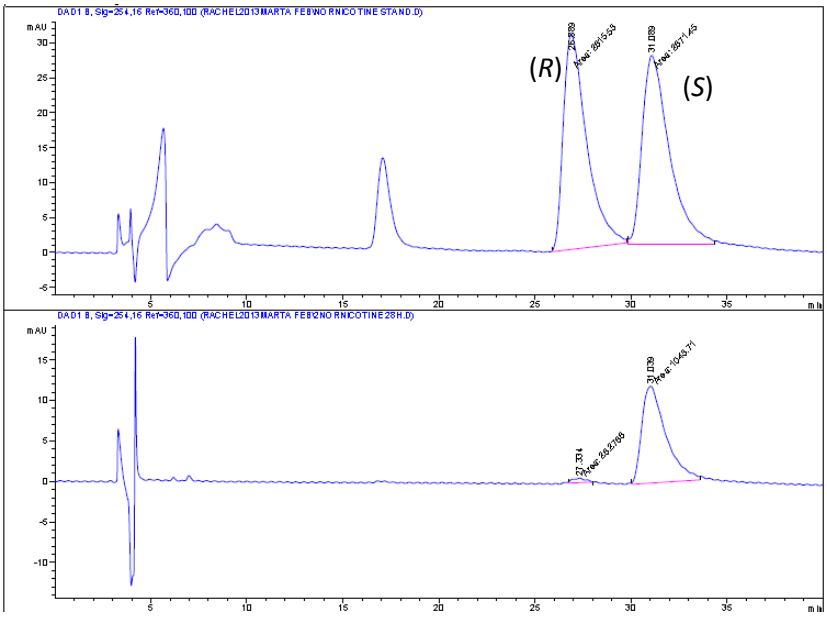
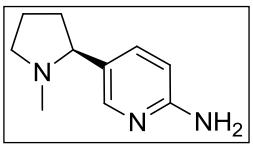


Figure S19. *Chiral phase HPLC trace of* (*rac*)-(*7*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*7*) (*top*) *and the deracemisation product* (*S*)-(*7*) (*bottom*).

4.3.3 (S)-6-Amino nicotine (8)



The biotransformation was carried out with (*rac*)-(8). After 3 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 98:2 + 0.1% DEA, flow rate 1.5 mL / min^{-1}; *Rt* [(*S*)] = 86.2 min, *Rt* [(*R*)] = 91.9 min. The absolute stereochemistry was assigned by analogy to 6-hydroxy nicotine.

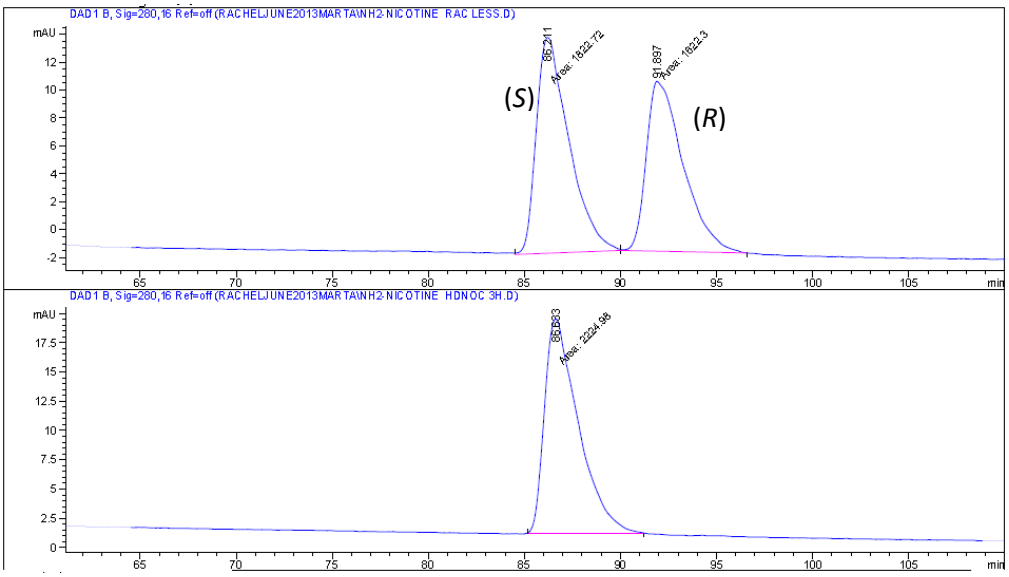
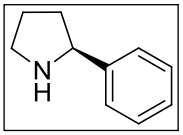


Figure S20. *Chiral phase HPLC trace of* (*rac*)-(*8*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*8*) (*top*) *and the deracemisation product* (S)-(*8*) (*bottom*).

4.3.4 (S)-2-Phenylpyrrolidine (12)



The biotransformation was carried out with (*rac*)-(12). After 48 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® ®IC column; flowrate 1 mL / min; UV 265 nM; eluent= hexane/iPrOH 90:10 + 0.1% DEA; *Rt* [(*R*)] = 7.5 min, *Rt* [(*S*)] = 8.2 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer).[2,11]

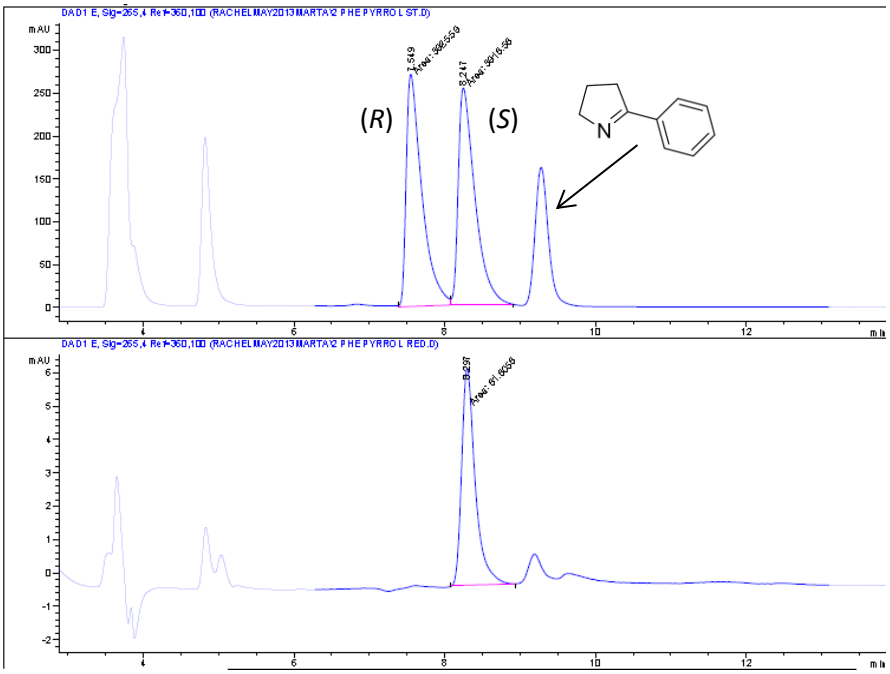
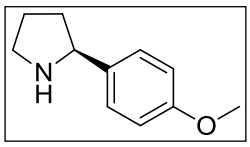


Figure S21. *Chiral phase HPLC trace of* (*rac*)-(*12*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*12*) (*top*) *and the deracemisation product* (S)-(*12*) (*bottom*).

4.3.5 (S)-2-(p-Methoxyphenyl)-pyrrolidine (13)



The biotransformation was carried out with (*rac*)-(13). After 44 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IE column; flowrate 1 mL / min; UV 265 nM; eluent= hexane/iPrOH 90:10 + 0.1% DEA; *Rt* [(*R*)] = 12.6 min, *Rt* [(*S*)] = 13.9 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer) (unpublished data) and analogy with 2-phenyl pyrrolidine.

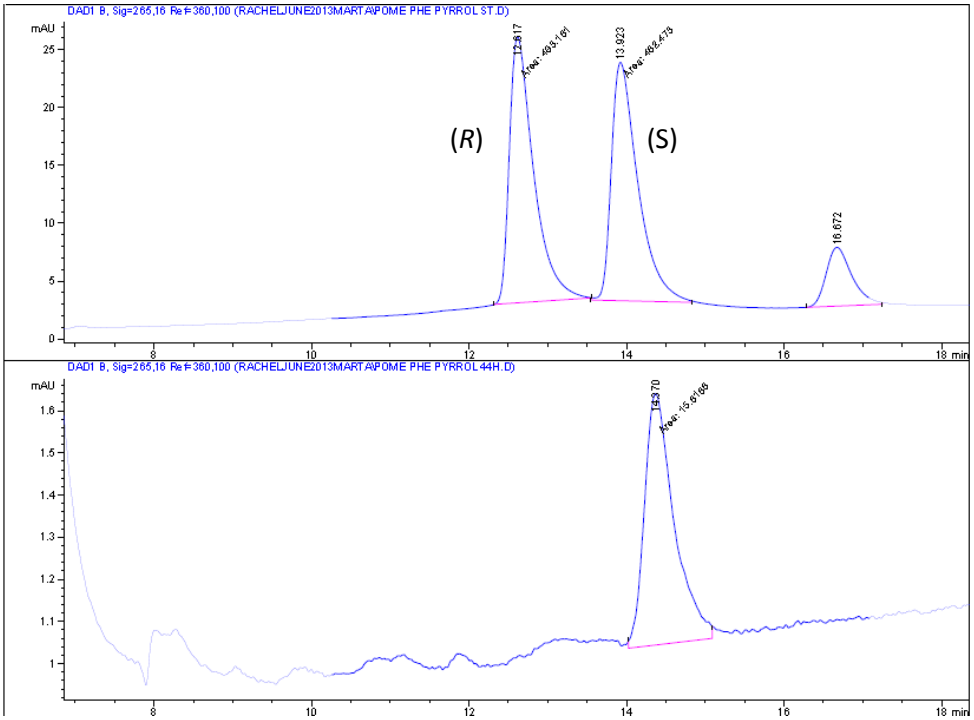
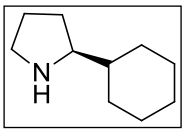
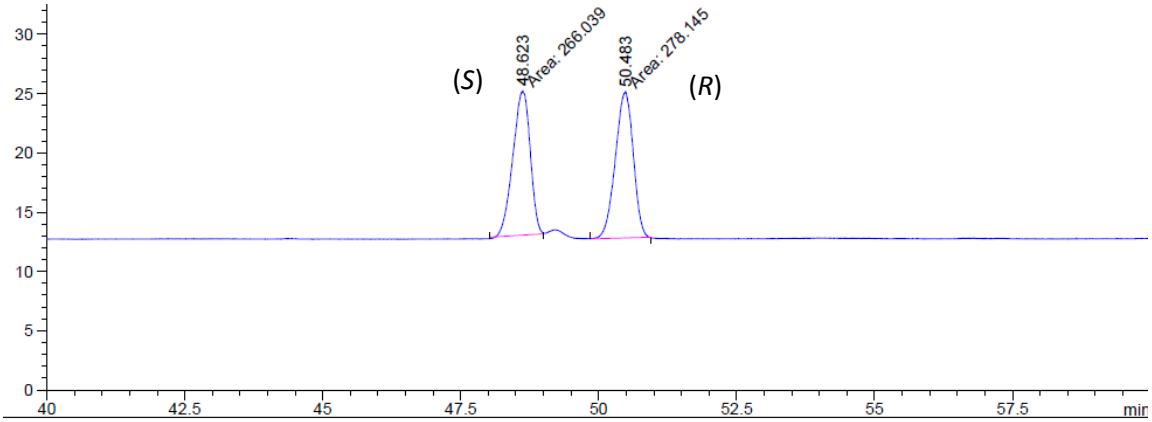


Figure S22. *Chiral phase GC trace of* (*rac*)-(*13*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*13*) (*top*) *and the deracemisation product* (S)-(*13*) (*bottom*).

4.3.6 (S)-2-Cyclohexylpyrrolidine (14)



The biotransformation was carried out with (*rac*)-(14). After 48 hours GC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was by GC on a chiral phase column. (14) was acetylated for GC analysis using trifluoracetic anhydride. Column used: Supelco β-DEX 325; carrier gas= Helium; injector temperature 250 ˚C; detector temperature (FID) 250 ˚C; flow 1.7 mL·min^{-1}; temperature program = isothermal 115 °C, *Rt* [(*S*)] = 48.6 min, *Rt* [(*R*)] = 50.5 min. The absolute stereochemistry was assigned by comparison of GC traces with the MAO-N biotransformation product ((*R*)-enantiomer).[10]



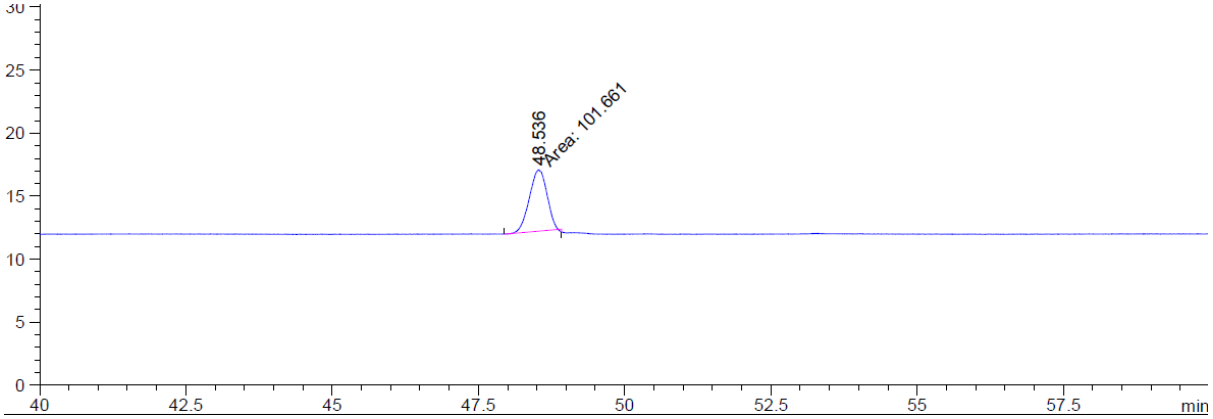
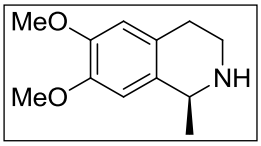


Figure S23. *Chiral phase GC trace of* (*rac*)-(*14*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound (14) (top) and the deracemisation product* (S)-(*14*) (*bottom*).

4.3.7 (S)-6,7-Dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline (15)



The biotransformation was carried out with (*rac*)-(15). After 48 hours HPLC analysis showed 81 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 280 nM; eluent= hexane/iPrOH 90:10 + 0.1% DEA; *Rt* [(*R*)] = 17.3 min, *Rt* [(*S*)] = 12.9 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer).[12]

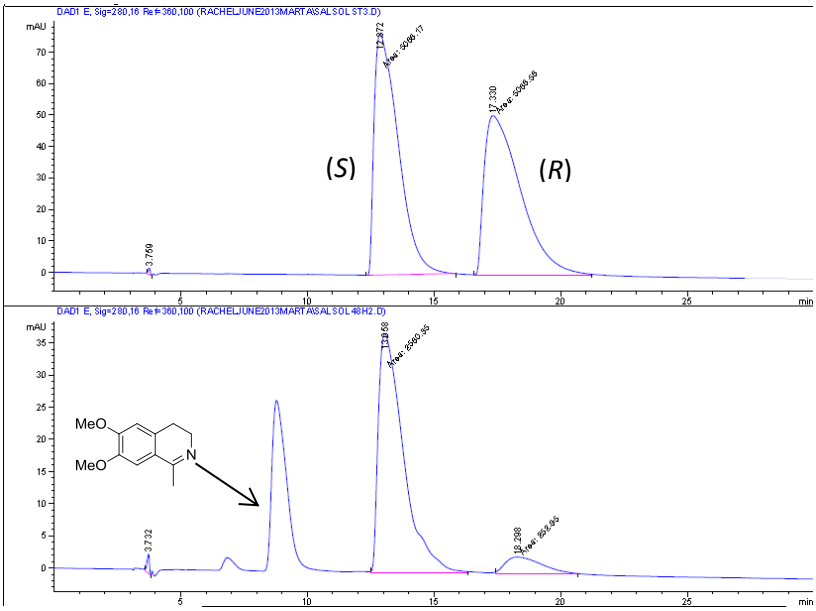
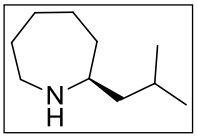


Figure S24. *Chiral phase HPLC trace of* (*rac*)-(*15*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*15*) (*top*) *and the deracemisation products, predominantly* (S)-(*15*) (*bottom*).

4.3.8 (S)-2-Isobutylazepane (18)



The biotransformation was carried out with (*rac*)-(18). After 24 hours GC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was by GC on a chiral phase column: (18) was acetylated for GC analysis using

acetic anhydride and triethylamine. Column used: Varian Chirasil-dex CB; carrier gas= Helium; injector temperature 200 ˚C; detector temperature (FID) 250 ˚C; flow 1.0 mL·min^{-1}; temperature program = from 90 °C to 150 °C with slope 10 °C·min^{-1} and from 150 °C to 200 °C with slope 1 °C·min^{-1}; *Rt* [(*R*)] = 13.1 min, *Rt* [(*S*)] = 13.6 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer) (unpublished data).

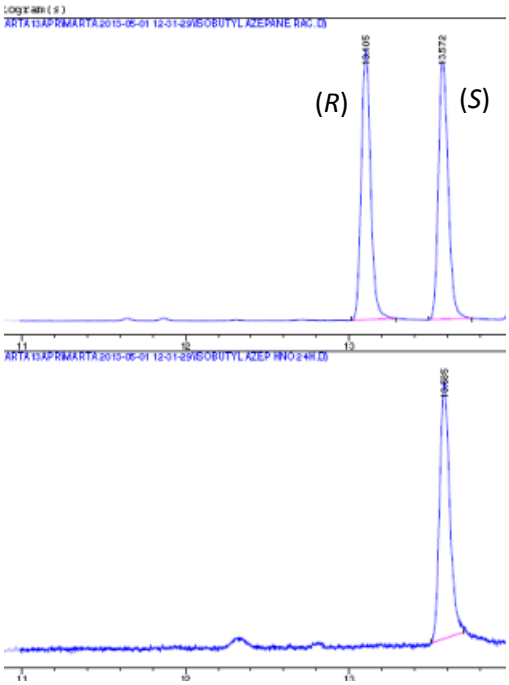
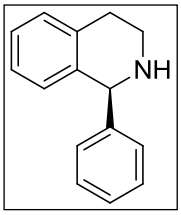
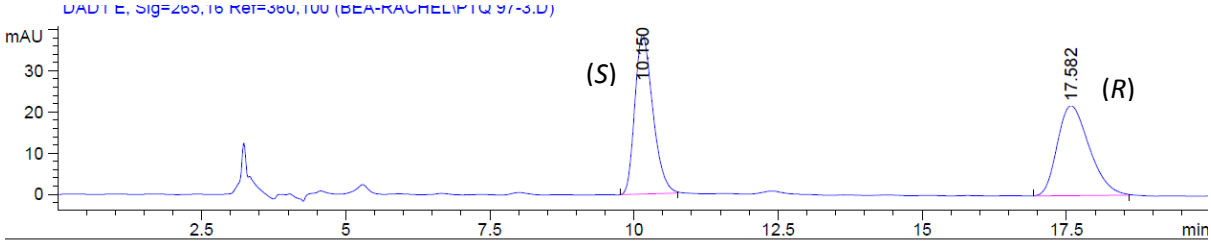


Figure S25. *Chiral phase GC trace of* (*rac*)-(*18*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*18*) (*top*) *and the deracemisation product* (S)-(*18*) (*bottom*).

4.3.9 (R)-1-Phenyl-1,2,3,4-tetrahydroisoquinoline (19)



The biotransformation was carried out with (*rac*)-(19). After 120 hours HPLC analysis showed 84 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 265 nM; eluent = hexane/iPrOH 97:3 + 0.1% DEA; *Rt* [(*R*)] = 17.6 min, *Rt* [(*S*)] = 10.2 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*S*)-enantiomer).[12] The apparent change in selectivity is merely due to the Cahn-Ingold-Prelog naming convention.



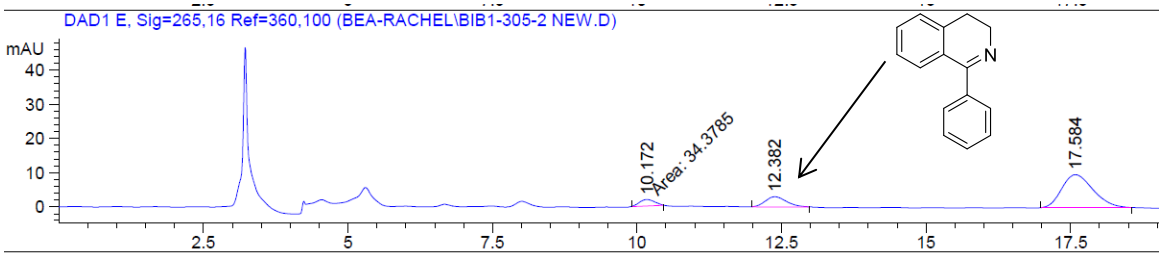
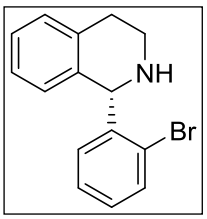


Figure S26. *Chiral phase HPLC trace of* (*rac*)-(*19*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*19*) (*top*) *and the predominant deracemization product* (R)-(*15*) (*bottom*).

4.3.10 (S)-1-(2-Bromophenyl)-1,2,3,4-tetrahydroisoquinoline (20) (oxidation only)



The biotransformation was carried out with (*rac*)-(20). After 48 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 265 nM; eluent= hexane/iPrOH 80:20 + 0.1% DEA; *Rt* [(*R*)] = 6.2 min, *Rt* [(*S*)] = 6.8 min. The absolute stereochemistry was assigned by analogy to 1-phenyl-1,2,3,4-tetrahydroisoquinoline. (N.B. The imine of this compound is difficult to reduce under the conditions used in this work hence only the oxidation was studied).

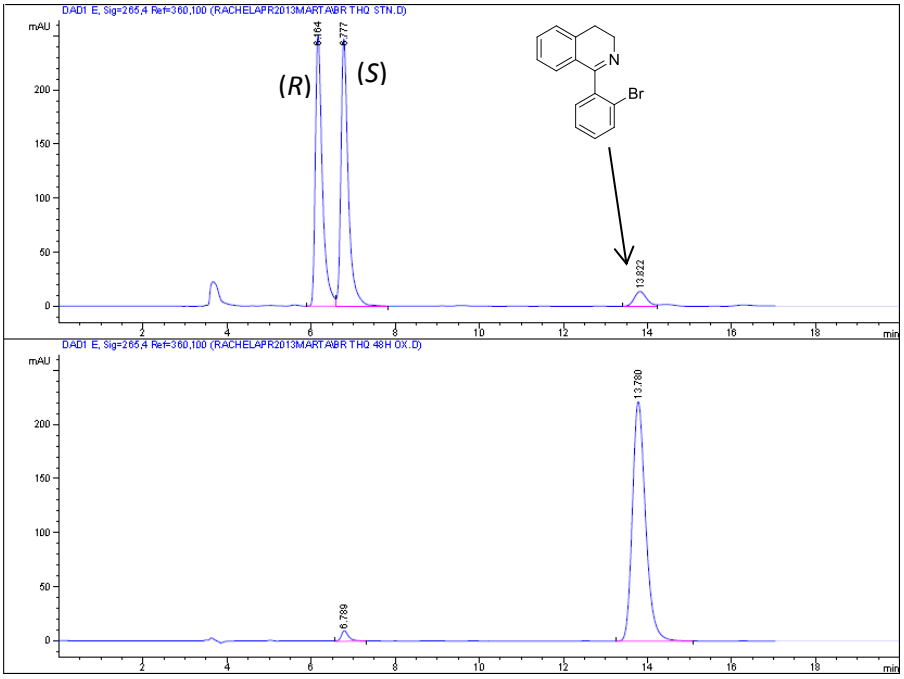
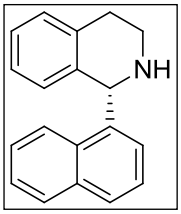


Figure S27. *Chiral phase HPLC trace of* (*rac*)-(*20*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*20*) (*top*) *and the oxidation products* (S)-(*20*) *and imine* (*bottom*).

4.3.11 (S)-1-(Naphthalen-1-yl)-1,2,3,4-tetrahydroisoquinoline (21) (oxidation only)



The biotransformation was carried out with (*rac*)-(21). After 24 hours HPLC analysis showed that the enzyme had some selectivity for the (*R*) enantiomer. Analysis was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flow rate 1 mL / min; UV 265 nM; eluent=

hexane/iPrOH 80:20 + 0.1% DEA; *Rt* [(*R*)] = 5.7 min, *Rt* [(*S*)] = 7.4 min. The absolute stereochemistry was assigned by analogy to 1-phenyl-1,2,3,4-tetrahydroisoquinoline. (N.B. The imine of this compound is difficult to reduce under the conditions used in this work hence only the oxidation was studied).

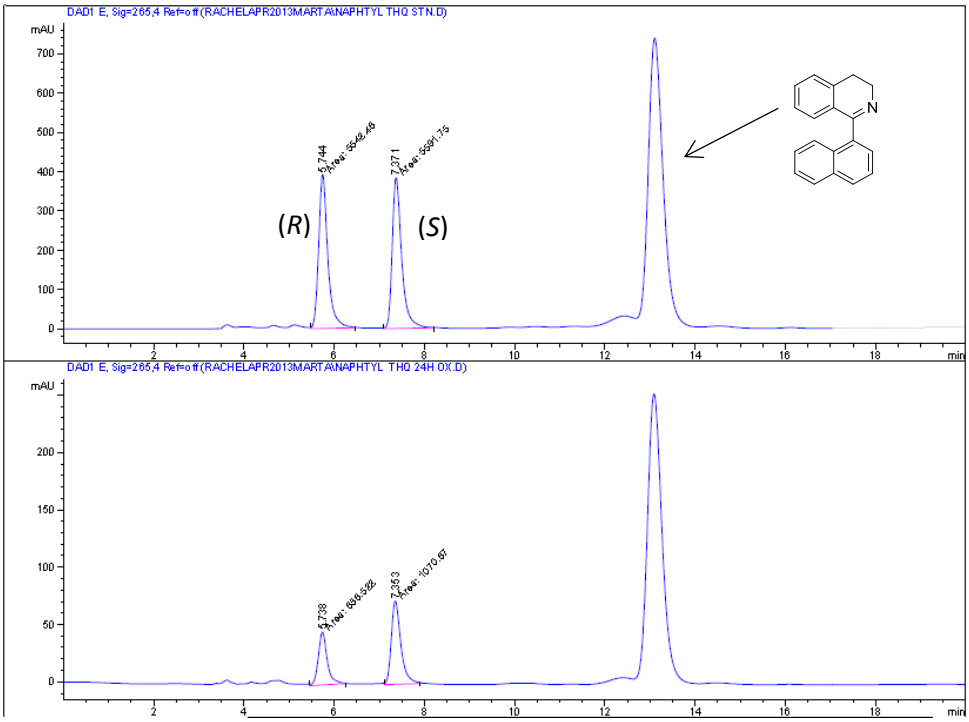
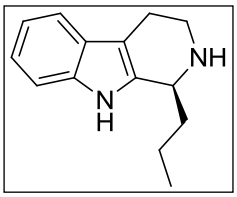


Figure S28. *Chiral phase HPLC trace of* (*rac*)-(*21*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*21*) (*top*) *and the oxidation products, predominantly* (S)-(*21*) *and imine* (*bottom*).

4.3.12 (S)-1-Propyl-4,9-tetrahydro-3H-β-carboline (22)



The biotransformation was carried out with (*rac*)-(22). After 28 hours HPLC analysis showed 29 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 mL / min; UV 254 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 22.9 min, *Rt* [(*S*)] = 14.1 min.

The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*S*)-enantiomer).[12]

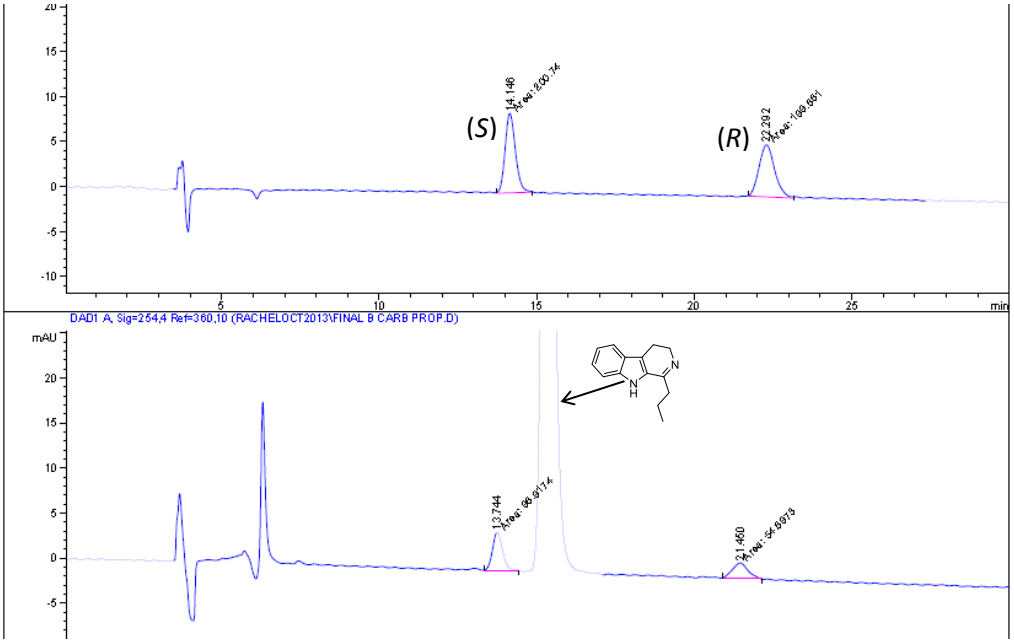
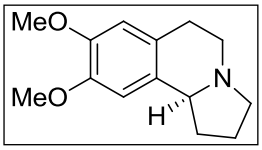


Figure S29. *Chiral phase HPLC trace of* (*rac*)-(*22*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*22*) (*top*) *and the predominant product,* (S)-(*22*) (*bottom*).

4.3.13 (S)-Crispine A (26)



The biotransformation was carried out with (*rac*)-(26). After 4 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 254 nM; eluent= hexane/iPrOH 90:10 + 0.1% DEA; *Rt* [(*R*)] = 15.8 min, *Rt* [(*S*)] = 10.3 min. The absolute stereochemistry was assigned by comparison of HPLC traces with the MAO-N biotransformation product ((*R*)-enantiomer).[4]

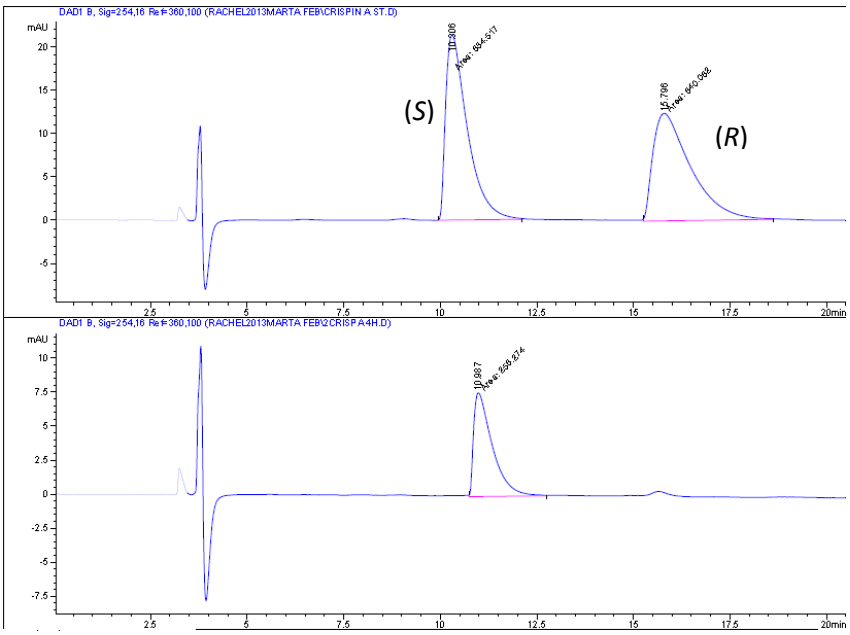
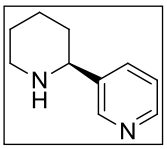


Figure S30. *Chiral phase HPLC trace of* (*rac*)-(*26*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*26*) (*top*) *and the deracemisation product,* (S)-(*26*) (*bottom*).

4.3.14 (S)-Anabasine (32)



The biotransformation was carried out with (*rac*)-(32). After 48 hours HPLC analysis showed 99 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL / min; UV 268 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 16.6 min, *Rt* [(*S*)] = 19.2 min. The absolute configuration was assigned by comparison of its optical rotation with the literature (see preparative procedure (4.4.9)).

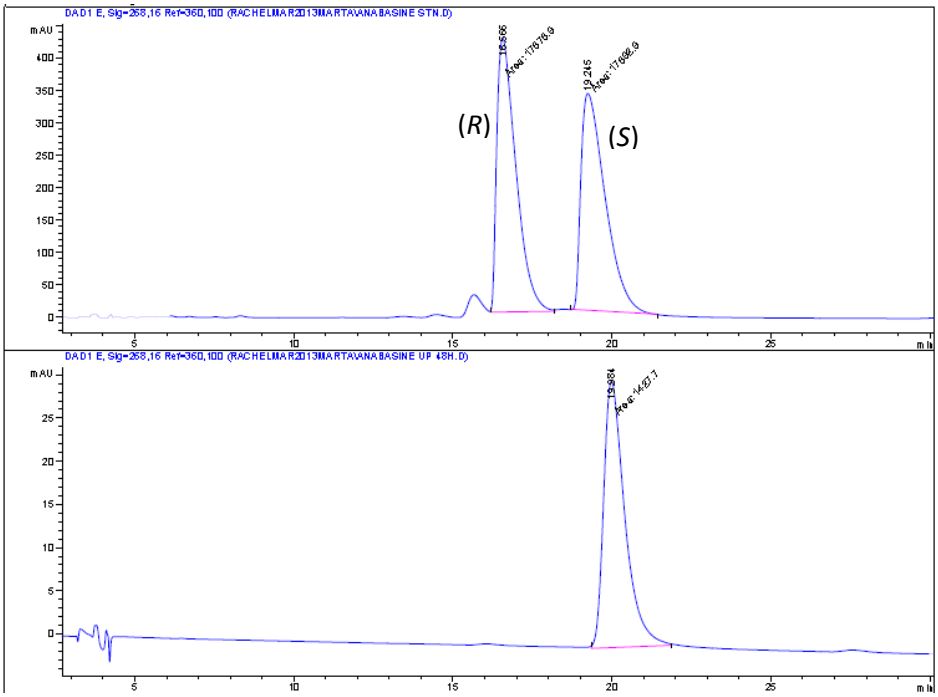
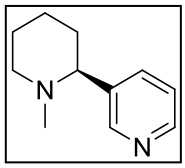
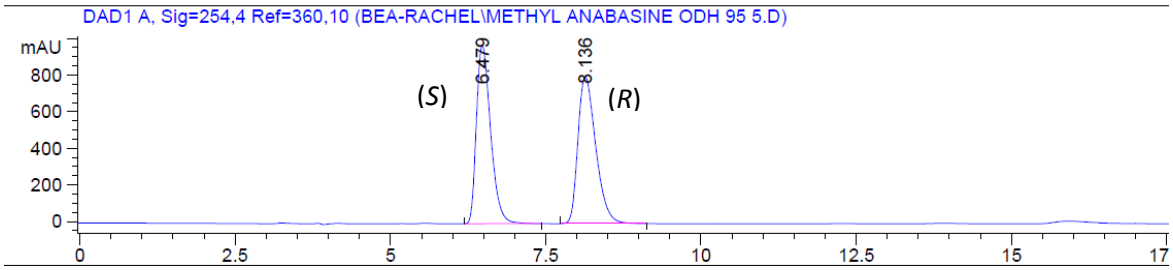


Figure S31. *Chiral phase HPLC trace of* (*rac*)-(*32*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*32*) (*top*) *and the deracemisation product* (S)-(*32*) (*bottom*).

4.3.15 (S)-3-(1-Methylpiperidin-2-yl)pyridine (33)



The biotransformation was carried out with (*rac*)-(33). After 48 hours HPLC analysis showed 97% *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALCEL® DAICEL OD-H column; flowrate 1 mL/min; UV 254 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*S*)] = 6.5 min, *Rt* [(*R*)] = 8.1 min. The absolute configuration was assigned by comparison of its optical rotation with the literature (see preparative procedure (4.4.10)).



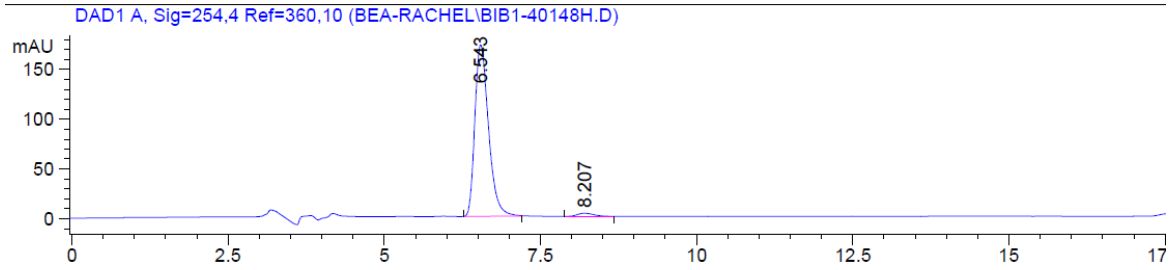
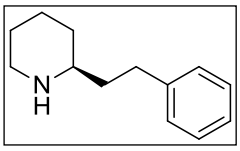


Figure S32. *Chiral phase HPLC trace of* (*rac*)-(*33*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*33*) (*top*) *and the deracemisation product* (S)-(*33*) (*bottom*).

4.3.16 (S)-2-(Phenylethyl) piperidine (34)



The biotransformation was carried out with (*rac*)-(34). After 48 hours HPLC analysis showed 90 % *e.e.* Determination of the enantiomeric excess was carried out by HPLC on a chiral phase column: CHIRALPAK® IC column; flowrate 1 ml / min; UV 268 nM; eluent= hexane/iPrOH 95:5 + 0.1% DEA; *Rt* [(*R*)] = 11.7 min, *Rt* [(*S*)] = 13.2 min. The absolute configuration was assigned by comparison of its optical rotation with the literature (see preparative procedure (4.4.11)).

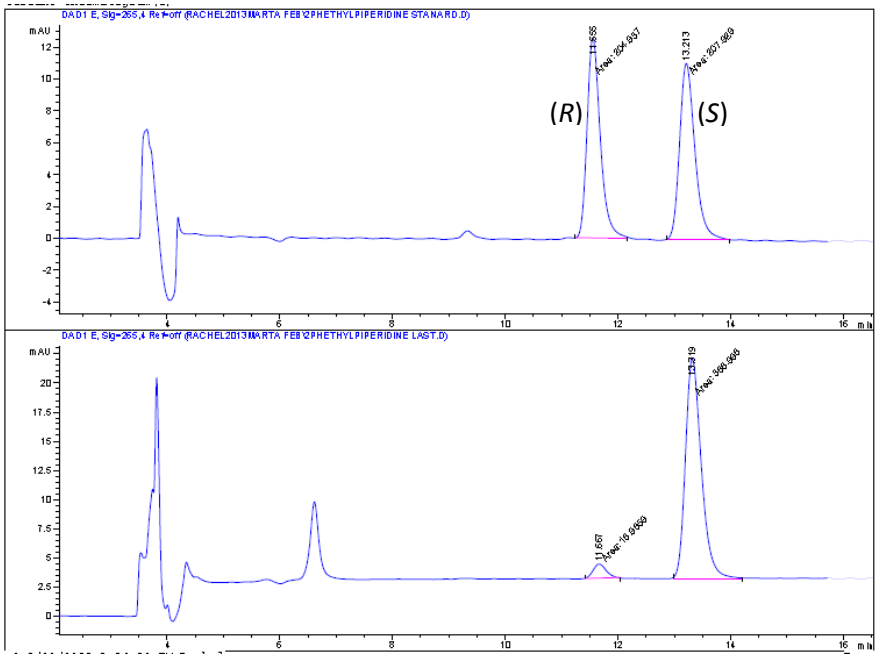


Figure S33. *Chiral phase HPLC trace of* (*rac*)-(*34*) *conversion with 6-HDNO E350L/E352D showing the signals of racemic compound* (*34*) (*top*) *and the deracemisation product* (S)-(*34*) (*bottom*).

4.3.17 Example comparison of opposite selectivities of MAO-N D5 and 6-HDNO

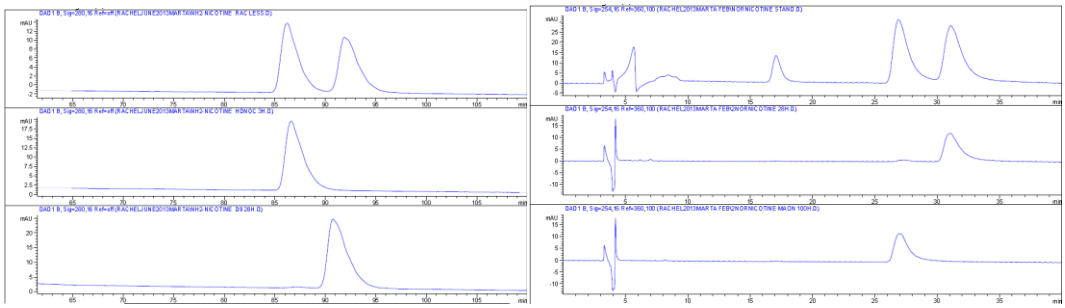


Figure S34. *Examples of opposite selectivities of MAO-N and 6-HDNO. LHS: Top: racemic 6-amino-nicotine, middle: product of deracemisation with 6-HDNO wt, bottom: product of deracemisation with MAO-N D5, RHS: Top: racemic nornicotine, middle: product of deracemisation with 6-HDNO, bottom: product of deracemisation with MAO-N D5.*

4.3.18 Example of solvent compatability of 6-HDNO E350L/E352D

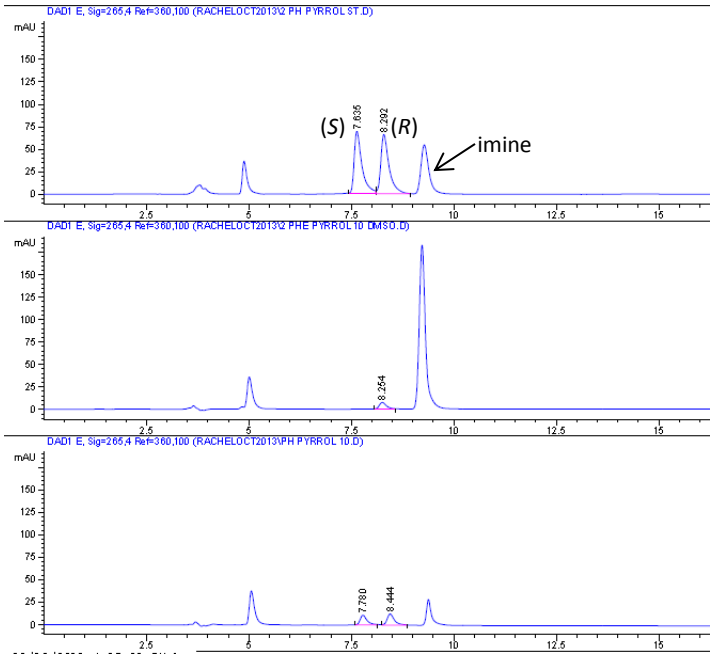


Figure S35*. Solvent compatability of 6-HDNO E350L/E352D with the substrate 2-phenyl pyrrolidine (oxidation only). Top: 2-phenyl pyrrolidine standard, middle: 10% DMSO – the enzyme has completely oxidised the (R)-enantiomer, bottom: 10% DMF – the enzyme has oxidized the (R)-enantiomer slightly (ee 6%).*

4.4 General preparative method

In a 500 mL screw cap bottle, the amine (2.5 mmol) and BH_3NH_3 (10 mmol, 4 eq.) were dissolved in potassium phosphate buffer (165 mL, 1 M, pH = 7.8). Cell pellet from *E. coli* cultures (5 g) containing HDNO was added to the solution. The bottle was placed in a shaking incubator and shaken at 37 °C and 250 rpm. When HPLC or GC analysis showed the conversion reached > 98 % and the amount of imine < 2%, aqueous NaOH (2 mL, 10 M) and

CH_2Cl_2 (200 mL) were added. The mixture was transferred in Falcon tubes and layers were separated by centrifugation (4000 rpm, 5 min.) and the aqueous phase was extracted again with CH_2Cl_2 (2 × 100 mL). The combined organic phases were dried with $MgSO_4$ and concentrated under reduced pressure.

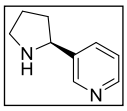
4.4.1 (S)-6-Hydroxy nicotine (1)

See 3.2.1

4.4.2 (S)-Nicotine

See 3.1.1

4.4.3 (S)-Nornicotine (7)

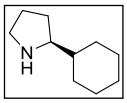


The reaction was set up as described in the preparative procedure using (*rac*)-nornicotine (50 mg, 0.335 mmol), BH_3NH_3 (42 mg, 1.34 mmol) and HDNO variant E350L/E352D (1 g) as biocatalyst. After 28 hours HPLC analysis showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/MeOH 9:1 + 1% Et_3N) to give (*S*)-nornicotine (95% *ee*, 30 mg, 60% yield).

$[\alpha]_D^{28}$ = -37.2 (*c* = 1.0, MeOH). (lit. $[\alpha]_D^{25}$ = -32.6 (c= 1, MeOH)).[13]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (1H, d, J =1.9 Hz), 8.41 (1H, dd, J=4.7, 1,5 Hz), 7.67 (1H, " dt, " J =7.9, 1.8 Hz), 7.18 (1H, dd, J=7.7, 4.8 Hz), 4.12 (1H, t, J=7.8 Hz), 3.17 – 3.11 (1H, m), 3.03 – 2.97 (1H, m), 2.21 – 2.13 (1H, m), 1.90-1.79 (1H, m), 1.69 – 1.60 (1H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.6, 148.4, 139.2, 134.3, 123.4, 60.0, 46.7, 34.0, 25.3.

4.4.4 (S)-2-Cyclohexylpyrrolidine (14)

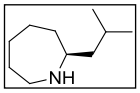


The reaction was set up as described in the preparative procedure using (*rac*)-2-cyclohexylpyrrolidine (50 mg, 0.326 mmol), BH_3NH_3 (41 mg, 1.30 mmol) and HDNO variant E350L/E352D (1 g) as biocatalyst. After 48 hours GC analysis of the acetylated compound showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/MeOH 9:1 + 1% Et_3N) to give (S)-2-cyclohexylpyrrolidine (99% *ee*, 36 mg, 71% yield).

$[\alpha]_D^{28}$ = -3.2 (*c* = 1.0, $CHCl_3$). [10]

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.10-3.03 (1H, m), 2.96 (1H, ddd, J=10.8, 8.6, 5.7 Hz), 2.77 – 2.33 (1H, m), 1.48 (1H, br s), 1.89 – 1.78 (2H, m), 1.74 – 1.61 (6H, m), 1.31 – 1.10 (5H, m), 1.1 – 0.9 (2H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 64.7, 46.5, 43.8, 31.3, 30.5, 29.7, 26.5, 26.2, 26.1, 25.2 ppm.

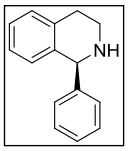
4.4.5 (S)-2-Isobutylazepane (18)



The reaction was set up as described in the preparative procedure using (*rac*)-2-isobutylazepane (50 mg, 0.32 mmol), BH_3NH_3 (40 mg, 1.29 mmol) and HDNO

variant E350L/E352D (1 g) as biocatalyst. After 24 hours GC analysis of the acetylated compound showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/MeOH 9:1 + 1% Et_3N); due to the nature of the product (not visible in the UV), the purification attempt with chromatography on silica gel did not give the pure compound.

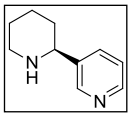
4.4.6 (R)-1-Phenyl-1,2,3,4-tetrahydroisoquinoline (19)



The reaction was set up as described in the preparative procedure using (*rac*)- 1-phenyl-1,2,3,4-tetrahydroisoquinoline (50 mg, 0.24 mmol), BH_3NH_3 (300 mg, 10 mmol) and HDNO variant E350L/E352D (3 g) as biocatalyst. After 120 hours HPLC analysis of the compound showed deracemisation up to 84% and the reaction was worked up. The compound was purified by column chromatography on silica gel (Hexane/EtOAc 4:6 + 1% Et_3N) to give (*S*)-1-phenyl-1,2,3,4-tetrahydroisoquinoline (84% *ee*, 43 mg, 86% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.19 (6H, m), 7.08 (2H, d, J =3.8 Hz), 6.99 – 6.95 (1H, m), 6.68 (1H, d, J =7.7 Hz), 5.05 (1H, s), 3.23 – 3.17 (1H, m), 3.06-2.95 (2H, m), 2.81 – 2.74 (1H, m), 1.65 (1H, br s) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 138.2, 135.4, 129.07, 129.02, 128.5, 128.1, 127.4, 126.3, 125.7, 62.1, 42.2, 29.8 ppm.

4.4.7 (S)-Anabasine (32)

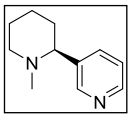


The reaction was set up as described in the preparative procedure using (*rac*)-anabasine (100 mg, 0.616 mmol), BH_3NH_3 (74 mg, 2.47 mmol) and HDNO variant E350L/E352D (2 g) as biocatalyst. After 72 hours HPLC analysis showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/cyclohexane 1:1) to give (*S*)-anabasine (99% *ee*, 56 mg, 56% yield).

$[\alpha]_D^{28}$ = -78.5 (*c* = 1.0, $CHCl_3$). (lit. $[\alpha]_D^{20}$ = -60.5 (c= 1, $CHCl_3$)).[5]

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (dt, *J*=2.2, 1H), 8.48 (dd, *J*=4.8, 1.7 Hz, 1H), 7.71 (dddd, *J*=7.8, 2.3, 1.7, 0.5 Hz, 1H), 7.29 – 7.20 (m, 1H), 3.63 (br dd, *J*=10.5, 2.8 Hz, 1H), 3.26 – 3.14 (m, 1H), 2.80 (ddd, *J*=11.6, 8.9, 2.9 Hz,), 1.98 – 1.83 (m, 1H), 1.81-1.74 (m, 1H), 1.71 – 1.62 (m, 1H), 1.57-1.44 (m, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9, 148.8, 140.9, 134.4, 123.7, 60.0, 47.8, 35.0, 25.9, 25.4.

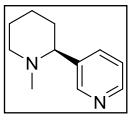
4.4.8 (S)-3-(1-Methylpiperidin-2-yl)pyridine (33) (wildtype)



The reaction was set up as described in the preparative procedure using (*rac*)-methyl anabasine (50 mg, 0.28 mmol), BH_3NH_3 (35 mg, 1.13 mmol) and wild-type HDNO wild-type (1 g) as biocatalyst. After 48 hours HPLC analysis showed deracemisation up to 84% and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/methanol 9:1 + 1% Et_3N) to give (*S*)-Methyl anabasine (84% *ee*, 30 mg, 60% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45-8.41 (2H, m), 7.62-7.60 (m, 1H), 7.18 (1H, dd, *J*=7.8, 4.7 Hz), 2.97 – 2.93 (1H, m), 2.74 (1H, dd, *J*=11.2, 2.8 Hz), 2.08 – 2.02 (m, 1H), 1.92 (s, 3H), 1.77 – 1.73 (m, 1H), 1.67 – 1.61 (m, 3H), 1.56 – 1.54 (1H, m), 1.32 – 1.28 (1H, m) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.3, 148.6, 140.2, 134.8, 123.6, 68.3, 57.4, 44.6, 36.0, 30.1, 26.0. 24.8.

4.4.9 (S)-3-(1-Methylpiperidin-2-yl)pyridine (33) (E350L/E352D variant)

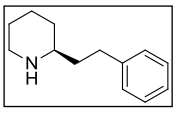


The reaction was set up as described in the preparative procedure using (*rac*)-methyl anabasine (50 mg, 0.28 mmol), BH_3NH_3 (35 mg, 1.13 mmol) and HDNO variant E350L/E352D (1 g) as biocatalyst. After 48 hours HPLC analysis showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/methanol 9:1 + 1% Et_3N) to give (*S*)-methyl anabasine (97% *ee*, 38 mg, 75% yield).

$[\alpha]_D^{28}$ = -105.8 (*c* = 1.0, MeOH). (lit. $[\alpha]_D^{20}$ = -132.5 (c= 0.8, MeOH)). [7]

For NMR data see 4.4.8.

4.4.10 (S)-2-(Phenylethyl) piperidine (34)



The reaction was set up as described in the preparative procedure using (*rac*)- 2-(phenylethyl) piperidine (100 mg, 0.528 mmol), BH_3NH_3 (63 mg, 2.1 mmol) and HDNO variant E350L/E352D (3 g) as biocatalyst. After 72 hours HPLC analysis showed full deracemisation and the reaction was worked up. The compound was purified by column chromatography on silica gel (EtOAc/cyclohexane 1:1) to give the (*S*)-2-(phenylethyl) piperidine (99% *ee*, 83 mg, 83% yield).

$[\alpha]_D^{25}$ = -14.7 (*c* = 1.0, MeOH). (lit. $[\alpha]_D^{20}$ = -11.3 (*c* = 0.95, MeOH)).[14-15]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.28 (m, 1H), 7.22 – 7.14 (m, 3H), 3.20 – 2.93 (m, 1H), 2.77 – 2.54 (m, 3H), 2.49 (dtd, *J*=8.9, 6.4, 2.5 Hz, 1H), 1.91 – 1.75 (m, 1H), 1.75 – 1.52 (m, 6H), 1.49 – 1.23 (m, 2H), 1.21 – 1.02 (m, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.6, 128.6, 128.5, 126.0, 56.7, 47.4, 39.4, 33.1, 32.5, 26.8, 25.0.

5. DNA and protein methods

5.1 Cloning

The gene for 6-HDNO from *Arthrobacter nicotinovorans* was codon optimised for expression in *E. coli* and synthesised by GeneArt® (Life Technologies). The gene was cloned into the vector pET16b (which contains a His_{10} tag to simplify purification) using NdeI/BamHI (restriction enzymes from New England Biolabs) restriction sites and transformed into *E. coli* BL21 (DE3) (New England Biolabs).

5.2 Expression

Overnight cultures were prepared by inoculating ~ 8 mL of LB containing 100 µg / mL ampicillin with a single colony and growing for 16 hours at 37 °C at 250 rpm. The overnight

culture was then added to a flask containing 800 mL autoinduction media[15] and 100 µg / mL ampicillin and grown for 48 hours at 25 °C with shaking at 200 rpm. Cells were spun down and stored as pellets at -20 °C. These were used either for purification or for biotransformations.

5.3 Purification

For purification the cells were thawed, resuspended in Buffer A (~1 g cell / 3 mL) and lysed first by adding lysozyme (Sigma) (1 mg / mL) and incubating at 30 °C for 20 minutes followed by 2 rounds of sonication (20s on, 20s off, x12 cycles) using a Soniprep 150 (MSE UK Ltd.). Cell debris was removed by centrifugation at 18000 rpm for 20 minutes. The lysate was loaded onto a HisTrap 1mL FastFlow column (GE Healthcare) and the column washed with 10 mL buffer A before the protein was eluted with a gradient of 0 – 100% buffer B over 30 minutes using an AKTA. Fractions corresponding to the peak at 280 nM were checked for purity by SDS-PAGE (Tris/glycine 4 – 20% Tris-Hepes gel, NuSep) and those fractions which were considered > 95% pure (Figure S36) were concentrated and buffer exchanged into 0.1 M phosphate buffer, pH 8.0, by spin column (Vivaspin 6, 30kDa cutoff, GE Healthcare). Purified protein was stored at 4 °C until use (typically 24 hours later). Buffer A: 100 mM Potassium phosphate pH 7.6 + 300 mM NaCl + 20 mM imidazole. Buffer B: 100 mM KPi pH 7.6 + 300 mM NaCl + 1 M imidazole. Specific activity was calculated at 30 °C, 100 mM pH 8.0 KPi buffer where U = µmol susbtrate consumed / min.

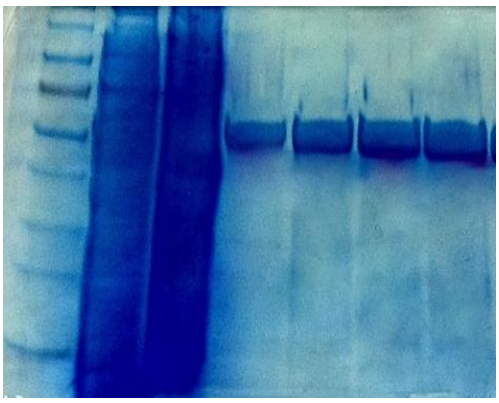


Figure S36*: Pure fractions of 6-HDNO obtained by affinity chromatograph* (*Lane 1: marker, Lane 2: load, Lane 3: flow-through, Lanes 4-7: pure 6-HDNO*).

5.4 Solid phase screen

The solid phase screen was used both to screen libraries and to screen a panel of substrates for activity. *E. coli* BL21 (DE3) was transformed with either a library or the wildtype or a single mutant. The transformation reaction was plated on a membrane (HyBond) on LB containing 100 µg / mL ampicillin and grown overnight at 30 °C. The membrane was then transferred to a second LB plate containing 100 µg / mL ampicillin and 1 mM IPTG and the protein expression induced for 4 hours at 30 °C after which membranes were kept frozen at -20 °C until use. Membranes were freeze-thawed three times (using liquid N_2) before being placed on filter paper containing 0.1 mg / mL Horse-radish peroxidase (HRP) (Sigma) in pH

8.0, 0.1 M potassium phosphate buffer. The membranes were left at room temperature for 1 hour. This ensured removal of any cellular H_2O_2. The membrane was the transferred to another filter paper containing a solution of 0.1 mg / mL HRP, 3,3'-Diaminobenzidine (DAB) (1 tablet per 15 mL, SigmaFast, Sigma), and 20 mM substrate. Colonies which turned dark red/brown indicated that the expressed protein was active on the substrate. For screening a panel of compounds, the membrane was cut into smaller pieces and each piece placed on a filter paper soaked in a different substrate (see Figure S37). In the case of screening libraries, colonies that changed colour were picked, added to 5 mL LB / amp and grown overnight, 37 °C, 250 rpm for extraction of the plasmid DNA by mini-prep (QIAPrep, Qiagen. The plasmids were then sequenced (GATC) using pET-RP and T7 primers to determine the mutation responsible for the activity.

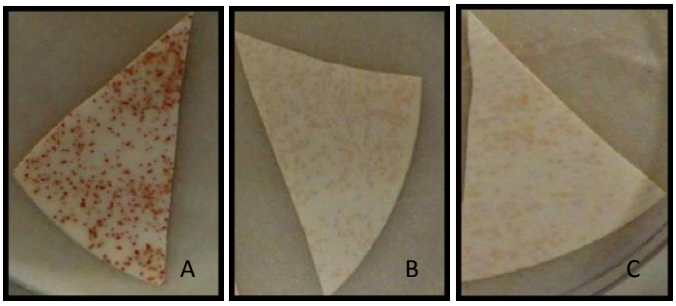


Figure S37*: A*) *20 mM* (R)-*nicotine, B*) *20 mM* (S)-*nicotine, C*) *no substrate*
E. coli *BL21* (*DE3*) *colonies expressing 6-hydroxy-D-nicotine oxidase on filter paper soaked in 0.1 mg / mL HRP, DAB and substrate.*

5.5 Libraries

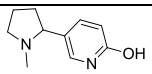
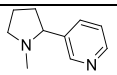
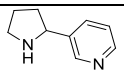
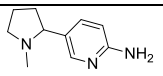
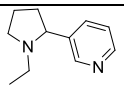
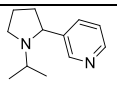
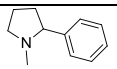
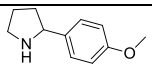
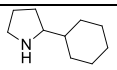
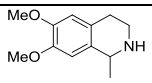
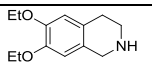
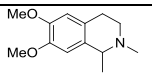
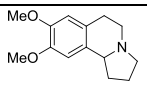
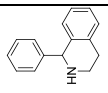
Libraries were made by designing primers at the appropriate regions and following the protocol from the "QuikChange II Site-Directed Mutagenesis Kit" (Agilent Technolgies) except that their Pfu polymerase was replaced with Phusion polymerase (NEB) and their dNTPs replaced with dNTPs from Roche. E350/E352 forward primer = 5'gcg tta aac tgn nka ttn nkg gta tgc cgt ttg g3', reverse primer = 5'cca aac ggc ata ccm nna atm nnc agt tta acg c3'. L373/L375 forward primer = 5'gca atg ggt gtt nnk gca nnk gca gaa tgg tca gg3', reverse primer = 5'cct gac cat tct gcm nnt gcm nna aca ccc att gc3'.

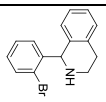
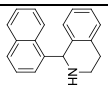
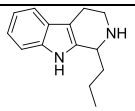
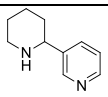
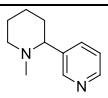
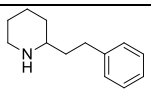
5.6 Kinetics

The initial rates were followed on a TECAN Infinite M200 spectrophotometer at 30 °C and 510 nM. A 1:1 ratio was assumed in the oxidation of substrate : production of hydrogen peroxide. The rate of production of hydrogen peroxide was detected by HRP (Type VI, Sigma) and 4-amino antipyrine/2,4,6-Tribromo-3-hydroxybenzoic acid dye (ε = 29400 L mol^{-1} cm^{-1}). The dye was made up by adding 100 µl 4-amino antipyrine (100 mg / mL) and 30 µL (20 mg / mL) 2,4,6-Tribromo-3-hydroxybenzoic acid to 10 mL of pH 8.0 potassium phosphate buffer. Substrates were dissolved in DMF to 0.5 M then diluted in buffer. Typically eight different substrates concentrations were examined (5 mM down to 0.005 mM). HRP was

dissolved in 0.1 Mpotassium phosphate buffer, pH 8.0, to give 0.2 mg / mL concentration. To a 96 well plate 50 µL substrate, 50 µL HRP and 50 µL dye were added. The assay was started by adding 50 µL purified enzyme (typically 0.1 – 2 mg / mL). Rate was plotted against substrate concentration and V_{max} and K_M values extracted using non-linear regression analysis with a fit to the equation $V_{max}.[S]/K_M+[S]$. The errors represent the 95% confidence interval of the variance in the fit. 6-HDNO is monomeric in solution.[9] Specific activities are given in Table 1.

Table 1. Specific activity of wildype and variant E350L/E352D

	Substrate	*Wt* / U	E350L/E352D / U
1		0.059	0.041
4		0.053	0.023
7		n.a.	0.023
8		0.14	0.055
9		n.d.	n.t.
10		n.d.	n.t.
11		n.d.	n.t.
13		n.a.	0.14
14		n.a.	0.00078
15		n.a.	0.15
16		n.a.	0.002
17		n.a.	0.053
18		n.a.	n.d.
26		0.28	0.44
19		n.a.	0.004

20		n.a.	0.016
21		n.a.	0.006
22		n.a.	0.01
32		n.a.	0.021
33		n.d.	0.00072
34		n.a.	0.016

n.t. = not tested, n.d.= not determined, n.a. = not active

5.7 Protein structure examination

The structure of 6-HDNO was examined by downloading the structure 2BVF from protein data bank (PDB) and using YASARA (http://www.yasara.org) as the visualization software.

6. References

[1] M. Amat, V. Elias, N. Llor, F. Subrizi, E. Molins, J. Bosch, *Eur. J. Org. Chem.* **2010**, 4017.

[2] C. J. Dunsmore, R. Carr, T. Fleming, N. J. Turner, *J. Am. Chem. Soc.* **2006**, *128*, 2224.

[3] A. E. Tachitschibabin,A. W. Kirssanow, *Berichte Der Deutschen Chemischen Gesellschaft* **1924**, *57*, 1163.

[4] I. Rowles, K. J. Malone, L. L. Etchells, S .C. Willies, N. J.Turner, N. J. *ChemCatChem* **2012**, *4*, 1259.

[5] T. Spangenberg, B. Breit, A. Mann, *Org. Lett.* **2009**, *11*, 261.

[6] K. Huang, F. G.Merced, M. Ortiz-Marciales, H. J.Melendez, W. Correa, M. De Jesus, *J. Org.Chem* **2008**, *73*, 4017.

[7] F. X. Felpin, S. Girard, G. Vo-Thanh, R. J. Robins, J. Villieras, J. Lebreton, *J. Org. Chem.* **2001**, *66*, 6305.

[8] M. Bruhmull, K. Decker, H. Mohler, *Eur. J. Biochem.* **1972**, *29*, 143.

[9] J. W. A.Koetter, G. E. Schulz, *J. Mol. Biol.* **2005**, *352*, 418.

[10] V. Koehler, Y. M. Wilson, M. Duerrenberger, D. Ghislieri, E. Churakova, T. Quinto, L. Knoerr, D. Haeussinger, F. Hollmann, N. J. Turner, N. J.; Ward, T. R. *Nature Chem.* **2013**, *5*, 93.

[11] R. Carr, M. Alexeeva, M. J. Dawson, V. Gotor-Fernandez, C. E. Humphrey, N. J.Turner, *ChemBioChem* **2005**, *6*, 637.

[12] D. Ghislieri, D. Houghton, A. P. Green, M. Pontini, S. C. Willies, N. J. Turner, *ACS Catalysis,* **2013,** doi: 10.1021/cs400724g.

[13] J. T. Ayers, R. Xu, L. P. Dwoskin, P. A. Crooks, *AAPS J.*, **2005**, *7*, E752.

[14] D. Ghislieri, A. P. Green, M. Pontini, S. C. Willies, I. Rowles, A. Frank, G. Grogan, N. J. Turner, N. J. *J. Am. Chem. Soc.* **2013**, *135*, 10863.

[15] A. R. Katritzky, G. F. Qiu, B. Z. Yang, P. J Steel,. *J. Org. Chem.* **1998**, *63*, 6699.

[16] T. Kiguchi, Y. Nakazono, S. Kotera, I. Ninomiya, T. Naito, T. *Heterocycles* **1990**, *31*, 1525.

[17] F. W Studier, *Protein Expression Purif.* **2005**, *41*, 207.