Electronic Supplementary Information

One-step Asymmetric Synthesis of (*R*)- and (*S*)-Rasagiline by Reductive Amination Applying Imine Reductases

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1. Imine Reductases

For this study, the IREDs listed in Table S1 were employed. The plasmids encoding these enzymes were used as published previously (Table S1).

Table S1 Imine reductases used in this study

IRED	Protein identifier	Organism	Reference
IR-1	WP_008741284.1	*Streptomyces sp. Mg1*	*1*
IR-3	WP_007131315.1	*Bacillales*	*1*
IR-4	WP_012921542.1	*Kribbella flavida DSM 17836*	*1*
IR-5	WP_023264430.1	*Cupriavidus sp. HPC(L)*	*1*
IR-6	WP_006236994.1	*Saccharomonospora xinjiangensis*	*1*
IR-9	WP_009737838.1	*Frankia sp. QA3*	*1*
IR-10	WP_011731218.1	*Mycobacterium smegmatis*	*1*
IR-11	WP_013733165.1	*Verrucosispora maris*	*1*
IR-12	WP_018958796.1	*Streptomyces sp. CNB091*	*1*
IR-13	YP_001108711.1	*Saccharopolyspora erythraea NRRL 2338*	*1*
IR-14	YP_005263141.1	*Nocardia cyriacigeorgica GUH-2*	*1*
IR-15	WP_026403156.1	*Actinomadura rifamycini*	*1*
IR-16	WP_026413575.1	*Actinomadura oligospora*	*1*
IR-17	WP_003931250.1	*Mycobacterium vaccae*	*1*
IR-18	WP_011857353.1	*Mycobacterium sp. JLS*	*1*
IR-19	WP_024839816.1	*Cellulosimicrobium cellulans*	*1*
IR-20	WP_006347397.1	*Streptomyces tsukubaensis*	*1*
IR-21	WP_025846174.1	*Paenibacillus ehimensis*	*1*
IR-22	WP_027751652.1	*Streptomyces sp. CNH287*	*1*
IR-23	WP_004001465.1	*Streptomyces viridochromogenes*	*1*
IR-32	WP_005335883.1	*Aeromonas veronii*	*2*
IR-33	WP_005354889.1	*Aeromonas veronii*	*2*
IR-Sgf3546	AB747175.1	*Streptomyces* sp. GF3546	*3, 4*
IR-Sgf3587	WP_015610874.1	*Streptomyces* sp. GF3587	*3, 5*
IR-Pel	WP_010497949.1	*Paenibacillus eglii* B69	*3*
IR-Sip	WP_009330409.1	*Streptomyces ipomoeae* 91-03	*3*
IR-Mma	ZP_00047589.2	*Magnetospirillum magnetotacticum* MS1	*3*
IR-Ppu	WP_010953707.1	*Pseudomonas putida* KT2440	*3*

2. Experimental Section

2.1. Chemicals

Substrates, solvents and product references (compounds **1a**-**1c**, **2a**, **2d**, **3b**, **3d**, **4c**) were sourced from Sigma Aldrich (St. Louis, USA), Enamines (Kiev, Ukraine), Alfa Aesar (Ward Hill, USA), Fluorochem Ltd (Hadfield, UK) and Active Scientific (Prien, Germany).

Table S2: Investigated ketones **1**-**4**, amines **a**-**i** and resulted products **1a**-**4d**

	a	b	c	d	e	f	g	h	i
1	1a	1b	1c	-	1e	1f	1g	1h	1i
2	2a	-	-	2d	-	-	-	-	-
3	-	3b	-	3d	-	-	-	-	-
4	-	-	4c	4d	-	-	-	-	-

2.2. Analytics

Apparent conversions of substrates **1**-**4** and **a**-**i** to products **1a**-**4d** were determined based on the % peak area of product and substrate ketones. For rasagiline, a calibration experiment for determining the response factors of the ketone and amine product was conducted. However, as the response factors differ less than 5 %, the % peak areas were used directly for calculating the apparent conversions.

Sample preparation: 40 µl of 10 M NaOH was added to 200 µl of the samples. The samples were extracted twice with 120 µl ethyl acetate and the combined organic phases were directly measured in GC (FID) or GC-MS (Gas chromatographs: GC-2010 Shimadzu, Kyoto, Japan). The columns (25 m x 0.25 mm) BPX-5 (SGE Analytical Science, Victoria, Australia) and FS-Hydrodex β-3p (Macheray-Nagel, Düren, Germany) were used.

The products of the preparative scale experiments were verified by ^{1}H- and ^{13}C-DEPT-NMR (Avance II 300, Bruker Cooperation, Billerica, USA). Deuterated chloroform was used as solvent. The product identity of **1e**, **1f**, and **1i** were verified by GC-MS (Figure S1).

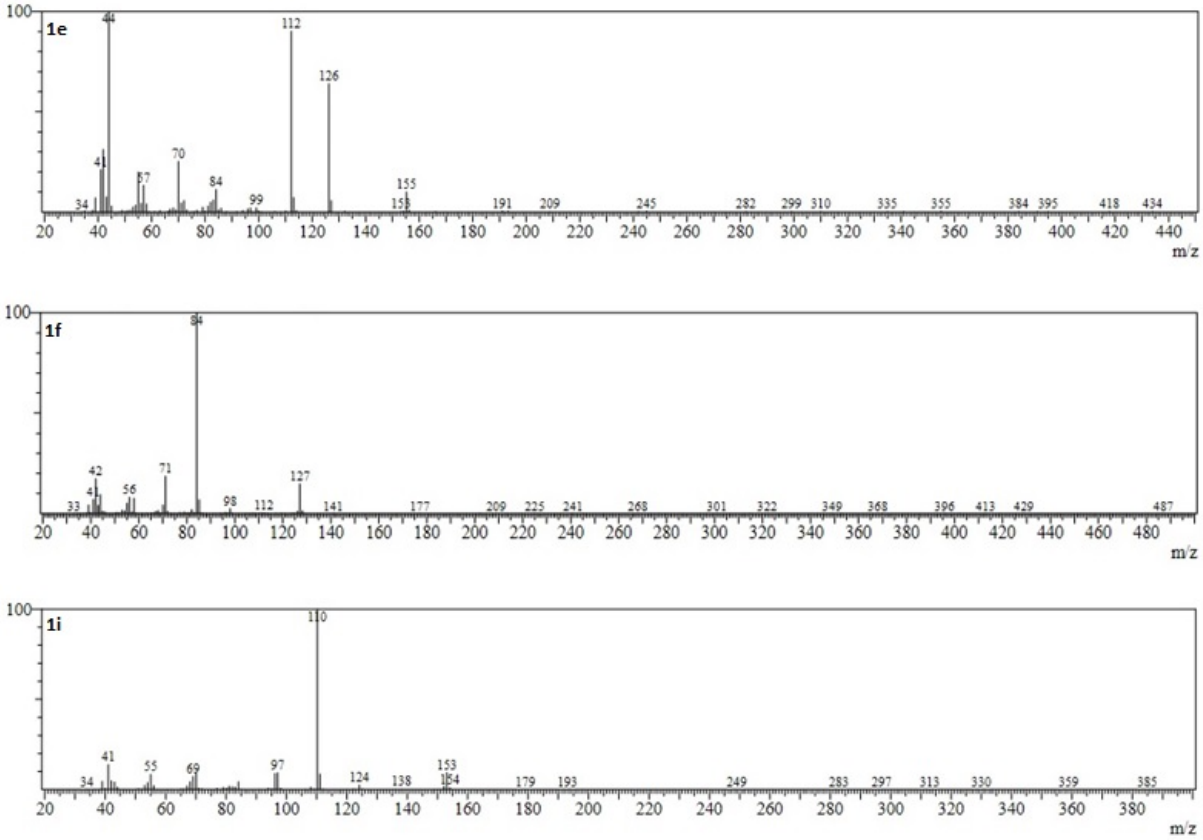


Figure S1: Mass spectra of the fragmented products **1e**, **1f** and **1i**.

Table S3: Summary of the GC analytics for the investigated substrates and products.

Compound	Column	Temperature program	Inlet pressure (kPa)	Inlet temperature (°C)	Retention time (min)
2a	BPX-5	75°C hold for 2 min, 150°C (10°C/min) hold for 2 min, 250°C (25°C/min)	82.7	280	**2**: 8.5 **2a**: 11.1
3b	BPX-5	75°C hold for 5 min, 110°C (5°C/min) hold for 5 min, 150°C (10°C/min) hold for 3 min, 220°C (15°C/min)	82.7	220	**3**: 15.5 **3a**: 17.8
4c	BPX-5	90°C hold for 5 min, 200°C (20°C/min) hold for 5 min, 260°C (5°C/min)	87.8	280	**4**: 15.5 **4a**: 14.5
1a-**1c**, **1e**-**1i**	BPX-5	60°C hold for 10 min, 220°C (10°C/min) hold for 5.5 min, 260°C (5°C/min)	78.9	300	**1**: 4.2, **1a**: 13.5, **1b**: 14.9, **1c**: 12.5, **1d**: 4.9, **1e**: 14.1, **1f**: 6.7, **1i**: 15.9

2d	BPX-5	60°C hold for 10 min, 100°C (10°C/min) hold for 10 min, 220°C (10°C/min) hold for 4 min	78.9	300	**2**: 23.1 **2d**: 20.3
3d	BPX-5	60°C hold for 10 min, 90°C (10°C/min) hold for 10 min, 220°C (10°C/min) hold for 4 min	78.9	300	**3**: 15.6 **3d**: 17.8
4d	BPX-5	80°C hold for 10 min, 220°C (10°/min) hold for 5 min	85.9	300°C	**4**: 22.1
(*S*)-**2a,** (*R*)-**2a**	FS-Hydrodex β-3p	90°C hold for 5 min, 180°C (10°C/min) hold for 10 min, 220°C (10°C/min) hold for 2 min	49	220	(*S*)-**2a**: 16.2 (*R*)-**2a**: 16.3
TPC-**3d**[1)]	BPX-5	150°C hold for 5 min, 170°C (10°C/min) hold for 10 min, 180°C (10°C/min) hold for 10 min, 300°C (10°C/min) hold for 5 min	109.8	300	26.6 and 27.4

[1)] Derivatisation with *N*-trifluoroacetyl-L-prolyl chloride, please see 2.4.1 for experimental details.

2.3. Substrate Screening

The screenings were performed with purified enzymes for a reaction time of 20 h for the initial and first screening while the incubation time was extended to 90 h for the second screening. The screenings were performed in 1 mL scale in 500 mM amine buffer, pH 9.5 (first and second screening: 200 mM), except methylamine was used at a concentration of 1 M. For the initial screening reactions, 20 mM ketone concentration was applied (20 mM in the first and second screening), 0.5 mg/mL purified enzyme and a NADPH recycling system consisting of 0.1 mg/mL of glucose dehydrogenase (Codexis GDH-105), 60 mM D-glucose, and 0.5 mM NADPH. The reaction was stopped with concentrated sodium hydroxide (end concentration 1.7 M). 240 µl of the solution was extracted twice with 120 µL ethyl acetate before analysis.

Enantiopure (*R*)- and (*S*)-rasagiline standards were used to determine the absolute configuration of the produced rasagiline.

The results for the screening are shown in Table 1, and a representative GC-chromatogram for each reaction is shown in Figure S2.

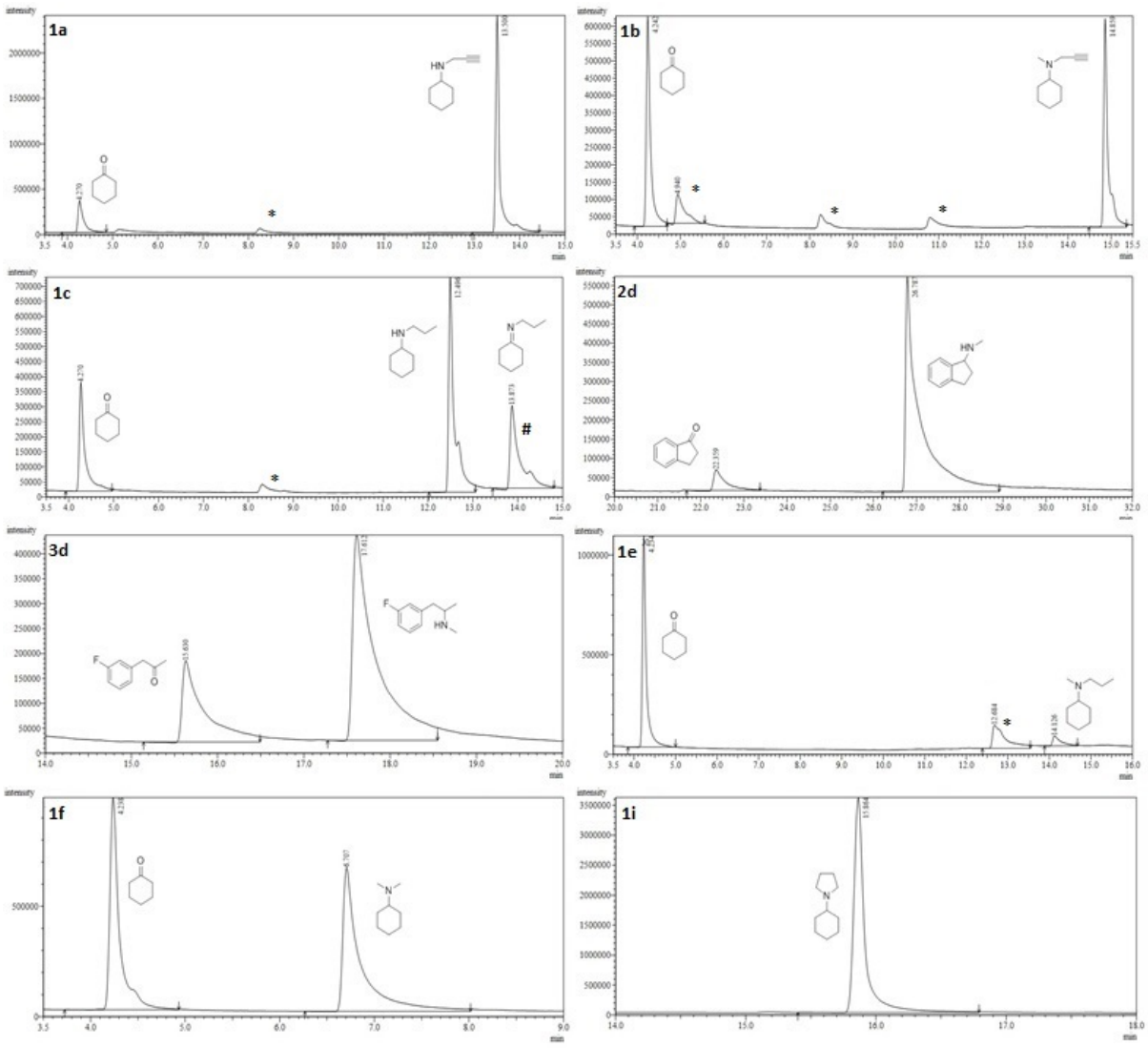


Figure S2: Representative GC-chromatograms for the substrate screening for product **1a**-**1c**, **1e**, **1f**, **1i**, **2d**, **3d** applying IREDs. *The structure of these impurities / side products could not be determined. #The MS-spectrum suggests that this peak corresponds to the imine. Imines were frequently observed during the reactions if the substrate ketones were not fully consumed.

Substrate 4 precipitated during time in the aqueous reaction solution (also in the absence of substrate amine), kinetic ^{1}H-NMR measurements were performed in D_2O to confirm that sufficient amounts of the substrate ketone is available within 20 h. The NMR-spectra at different time points (0 h, 5 h, 20 h) are shown in Figure S3a-c.

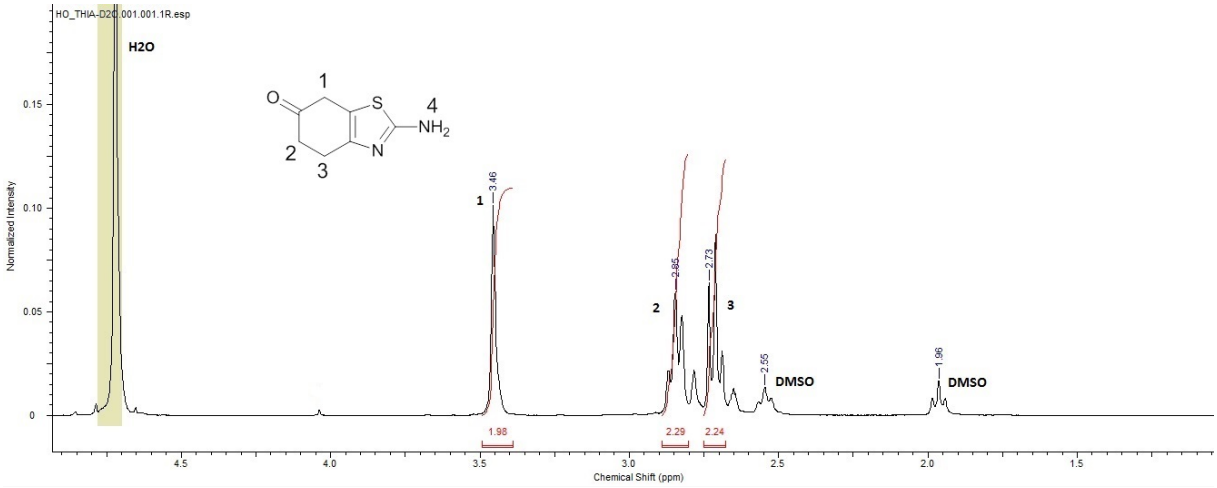


Figure S3a: ^{1}H-NMR spectrum for 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole (**4**) in 75% D_2O and 25% DMSO at time point **0 h.**

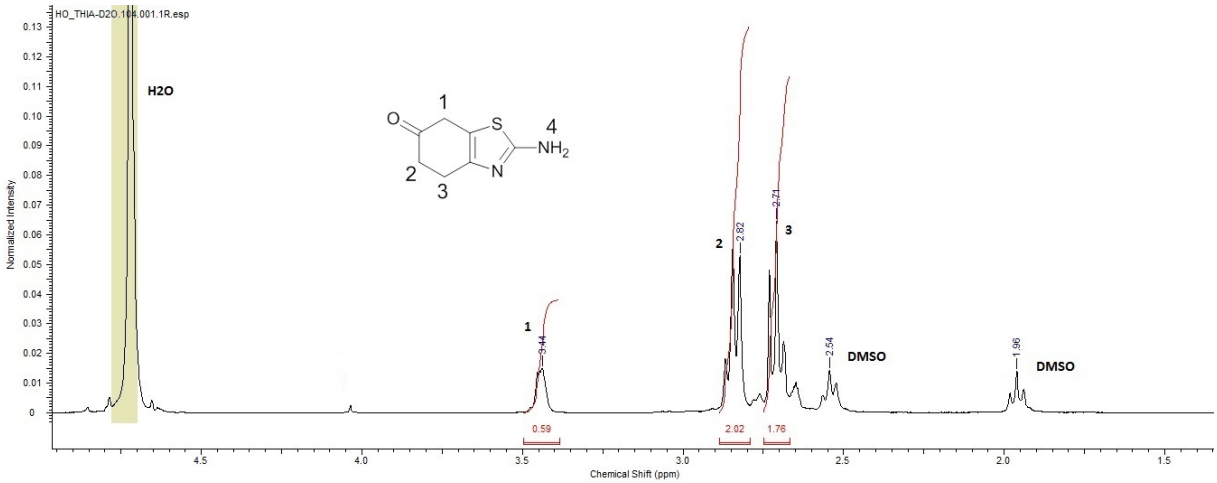


Figure S3b: ^{1}H-NMR spectrum for 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole (**4**) in 75% D_2O and 25% DMSO after **5 h.**

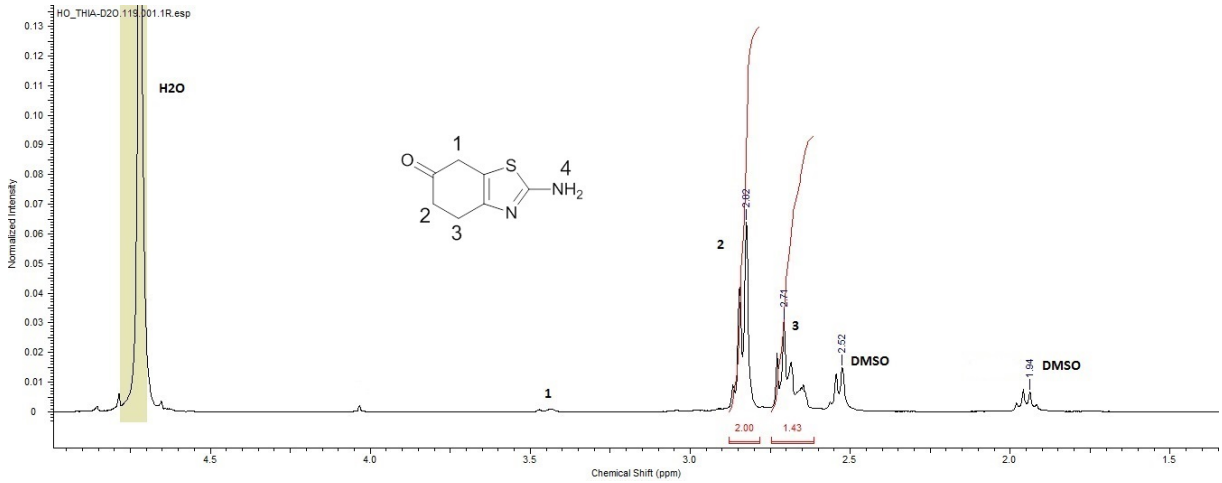
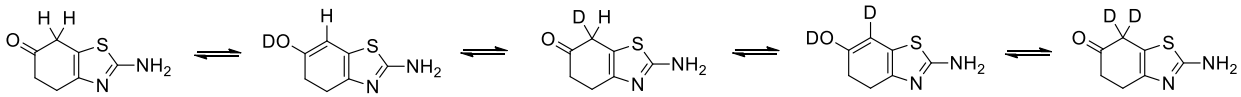


Figure S3c: ^{1}H-NMR spectrum for 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole (**4**) in 75% D_2O and 25% DMSO after **20 h.**

After 20 h, the substrate 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole (**4**) was detected in the ^{1}H-NMR spectrum with a slightly reduced concentration (as judged from signal intensities compared to the DMSO signal) compared to the first measurement (0 h); no side product was observed. 25 % deuterated DMSO was added for solubility reasons. The residual water content led to the occurrence of two DMSO signals, no signal for the amino group was visible. Furthermore the signal for the two protons on position 1 was decreasing during time. This can be explained by a ketone-enol tautomerization, where the protons at position 1 are exchanged by deuterons from the solvent D_2O (Scheme S3d).

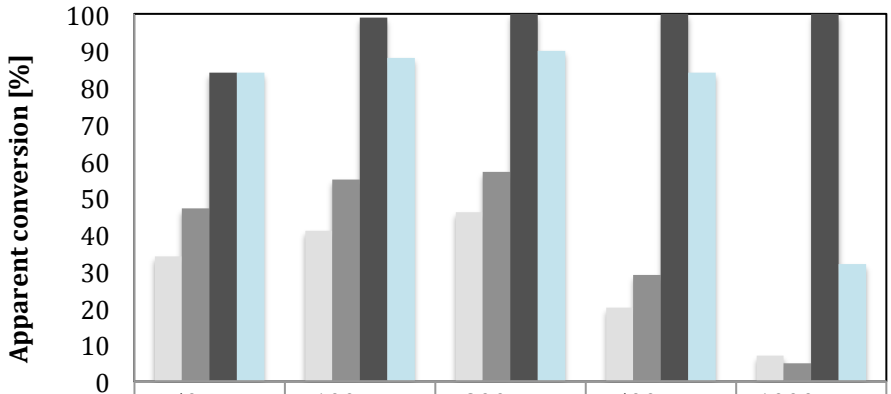


Scheme S3d: Ketone-enol tautomerization of 2-amino-6-oxo-4,5,6,7-tetrahydrobenzothiazole (**4**) in D_2O.

2.4 Preparative Reactions

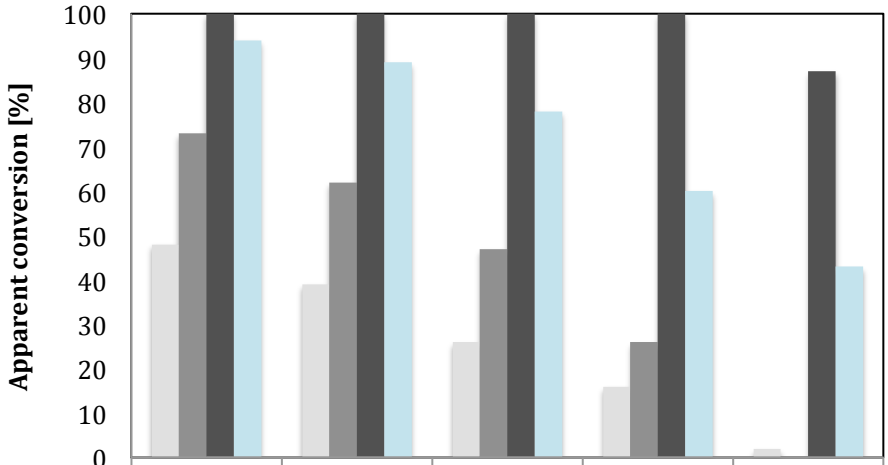
2.4.1 Reaction optimization

To optimize the preparative reactions, different reaction conditions were investigated to reach high conversion for the products: rasagiline (**2a**), methampethamine (**3d**) and *N*-methylpropargyl cyclohexanamine (**1b**). In small scale experiments ketone concentrations were varied from 5 mM up to 50 mM, amine concentrations from 50 mM up to 1000 mM, enzyme concentrations between 0.25 mg/mL and 2.5 mg/mL and samples were taken at different time points from 5 h to 168 h (Figure S4-S7). In order to compare the results, only one parameter was varied. The standard conditions were set as follows: 10 mM ketone, 200 mM (except for methylamine 1 M) amine buffer (pH 9.5), IRED 1.5 mg/mL, 60 mM glucose, 0.5 mM NADPH, 0.1 mg/mL glucose dehydrogenase (Codexis GDH-105)., reaction time: 90 h at 30°C.



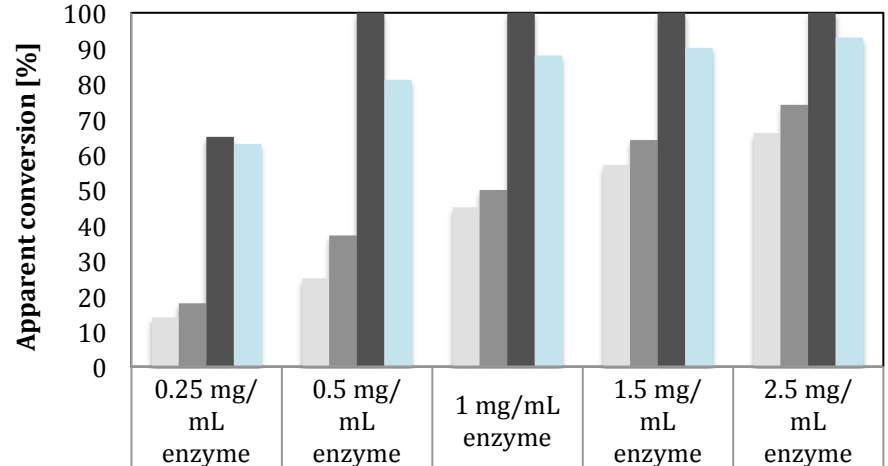
	50 mM amine	100 mM amine	200 mM amine	500 mM amine	1000 mM amine
(R)-rasagiline	34	41	46	20	7
(S)-rasagiline	47	55	57	29	5
4-fluoro methamphetamine*	84	99	100	100	100
N-methylpropargyl cyclohexanamine	84	88	90	84	32

Figure S4: Optimization of the substrate amine concentrations. Conversions after 90 h were measured by GC. *For 4-fluoro methamphetamine **3d,** apparent conversions of >80% are inaccurate due to a higher limit of quantification for the substrate ketone.



	5 mM ketone	10 mM ketone	20 mM ketone	30 mM ketone	50 mM ketone
(R)-rasagiline	48	39	26	16	2
(S)-rasagiline	73	62	47	26	0
4-fluoro methamphetamine*	100	100	100	100	87
N-methylpropargyl cyclohexanamine	94	89	78	60	43

Figure S5: Optimization of the substrate ketone concentrations. Conversions after 90 h were measured by GC. *For 4-fluoro methamphetamine **3d,** apparent conversions of >80% are inaccurate due to a higher limit of quantification for the substrate ketone.



	0.25 mg/ mL enzyme	0.5 mg/ mL enzyme	1 mg/mL enzyme	1.5 mg/ mL enzyme	2.5 mg/ mL enzyme
(R)-rasagiline	14	25	45	57	66
(S)-rasagiline	18	37	50	64	74
4-fluoro methamphetamine*	65	100	100	100	100
N-methylpropargyl cyclohexanamine	63	81	88	90	93

Figure S6: Optimization of the enzyme concentrations. Conversions after 90 h were measured by GC. *For 4-fluoro methamphetamine **3d,** apparent conversions of >80% are inaccurate due to a higher limit of quantification for the substrate ketone.

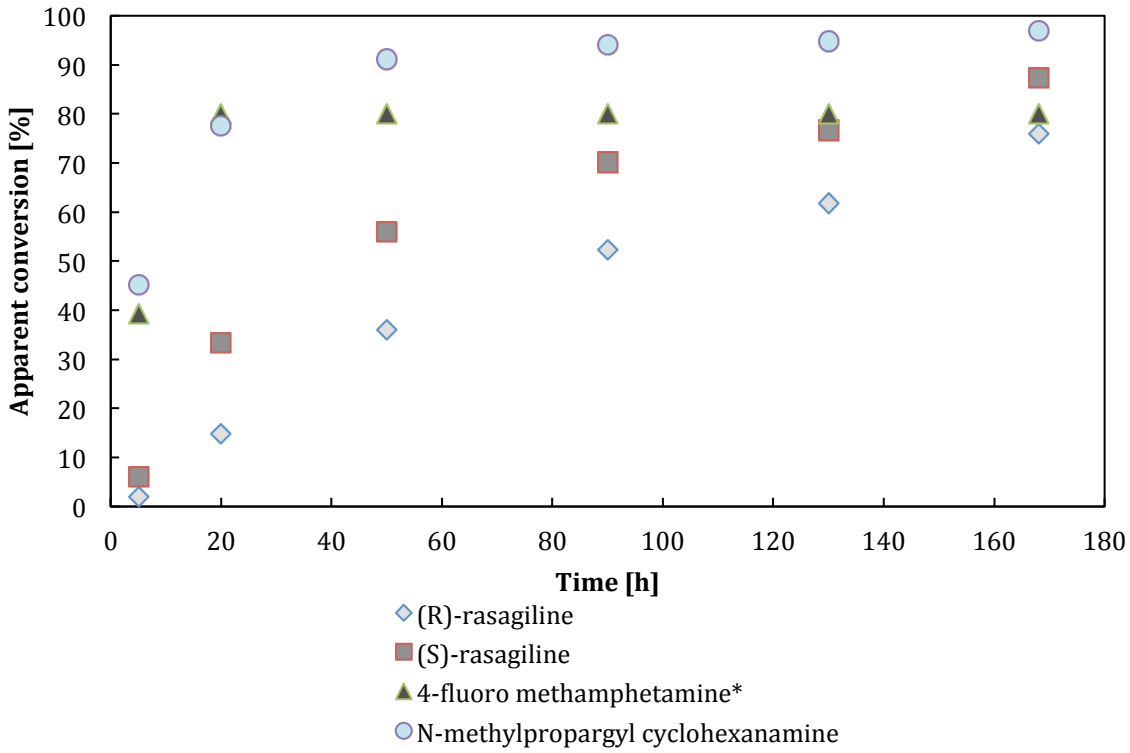


Figure S7: Time course of the reactions. Conversion over time to (*R*)-rasagiline **2a** (IR-14), (*S*)-rasagiline **2a** (IR-Sip), 4-fluoro methamphetamine **3d** (IR-Sip), and *N*-methylpropargyl cyclohexanamine **1b** (IR-14) was measured with GC. *For 4-fluoro methamphetamine **3d,** apparent conversions of >80% are inaccurate due to a higher limit of quantification for the substrate ketone. Conditions: 10 mM ketone, 200 mM (except for methylamine 1 M) amine buffer (pH 9.5), IRED 1.5 mg/mL, 60 mM glucose, 0.5 mM NADPH, 0.1 mg/mL glucose dehydrogenase (Codexis GDH-105)., reaction time: 90 h at 30°C.

2.4.1 Reaction Analysis

Under optimized conditions preparative reactions have been performed. The reactions were incubated for 7 days and the apparent conversions were detected by GC-MS. Enantiomeric excess (%ee) was detected for rasagiline and 4-fluoro methamphetamine by chiral GC measurement. 4-fluoro methamphetamine was derivatized prior to GC-MS analysis with *N*-trifluoroacetyl-L-prolyl chloride (TPC) to the amide TPC-**3d** according to an established literature protocol. Briefly, 100 µL of diluted amine (concentration ~ 20 mM) was reacted with 0.2 mL TPC and 4 µL triethylamine for 15 min at room temperature. 200 µL 6 M HCl was added and the organic phase was washed two times with water. The organic phase was dried with $MgSO_4$ and injected into the GC-MS. Full conversion was detected (absence of substrate amine in the chromatogram).[6]

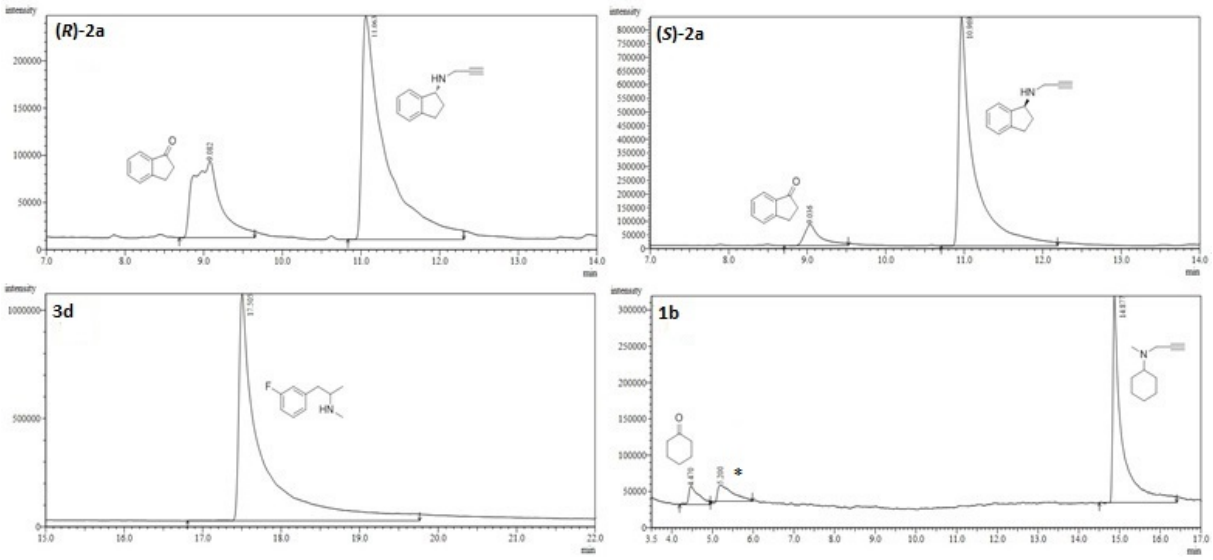


Figure S8: GC-MS chromatograms for calculation of the apparent conversion for preparative reactions catalyzed by IR-14 ((*R*)-**2a**, **1b**) and IR-Sip ((*S*)-**2a**, **3d**). *The structure of this impurity / side product could not be determined.

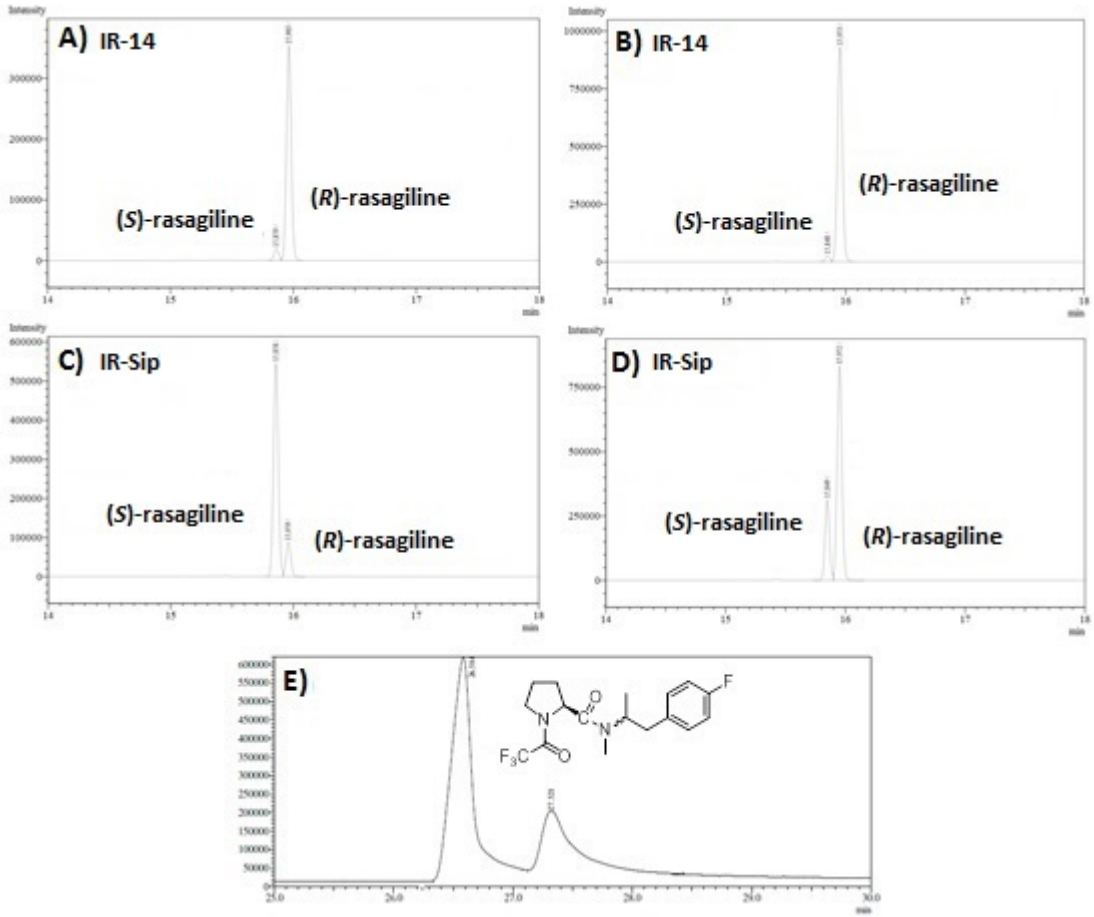


Figure S9: GC chromatograms for the detection of the enantiomeric excess of the preparative scale reactions to rasagiline and 4-fluoro methamphetamine. To assign the absolute configuration, the GC-samples were spiked with enantiopure (*R*)-rasagiline. (**A**) (*R*)-rasagiline catalyzed by IR-14 ee: 90%; (**B**) Same as (A) but spiked with (*R*)-rasagiline; (**C**) (*S*)-rasagiline catalyzed by IR-Sip ee: 72% (**D**) Same as (C) but spiked with (*R*)-rasagiline; (**E**) 4-fluoro methamphetamine catalyzed by Rir-Sip ee: 52%.

2.4.3 Workup and NMR

The products were isolated including following workup:

The remaining ketone was extracted after addition of concentrated HCl (final pH ~ 2) with 0.5 reaction volumes ethyl acetate (or DCM) for three times (precipitated enzyme was filtered). Subsequently, the amine product was extracted after addition of concentrated NaOH (final pH ~ 12) with ethyl acetate or DCM (half reaction volume) for three times. The organic phases were dried with magnesium sulfate (approx. 0.9 g), filtered, followed by an evaporation step to remove the remaining substrate amine (propargylamine: 85°C at 10 mbar for 25 min, *N*-methyl-*N*-propargylamin: 85°C at 10 mbar for 35 min, methylamine: 42°C at 700 mbar). Finally, 1.2 equivalents (referring to the product amine at 100 % conversion) of hydrogen chloride solution (2 M) in diethyl ether was added to the redissolved product to isolate the products as HCl-salts after solvent removed by evaporation.

To verify the products, ^{1}H-NMR and ^{13}C-NMR were recorded. The spectra are shown in Figure S10-S13. To investigate the purity of the isolated products a GC-scan measurement from 60°C to 350°C was performed (Figure S14) and revealed the followed purities: (*R*)-rasagiline: >95%, (*S*)-rasagiline: 91%, 4-fluoro methamphetamine 90%, *N*-methyl-*N*-propargyl cyclohexanamine: >95%.

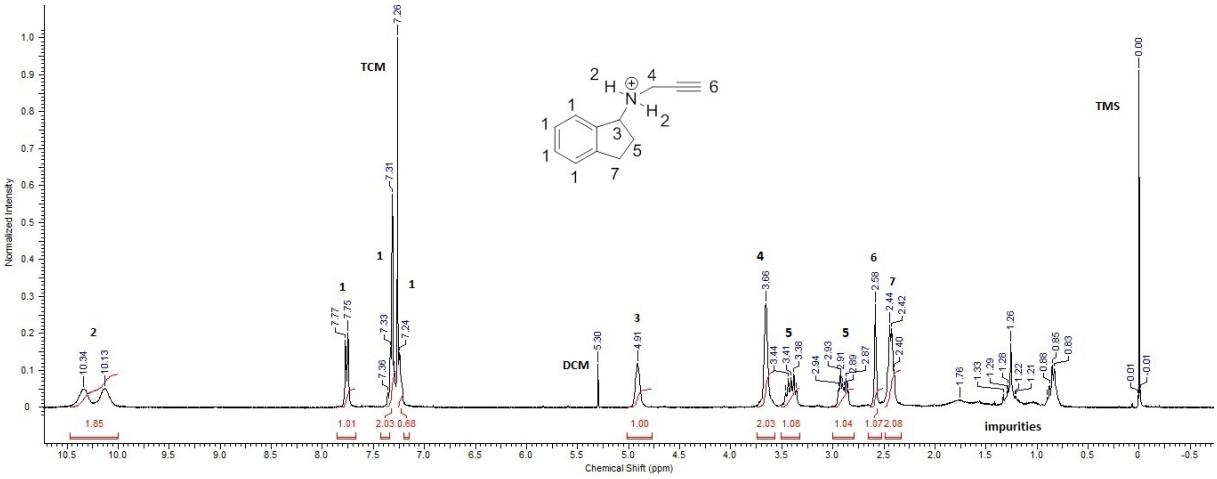


Figure S10a: ^{1}H-NMR spectrum of the isolated product (*R*)-rasagiline (**2a**) in chloroform synthesized by reductive amination applying IR-14.

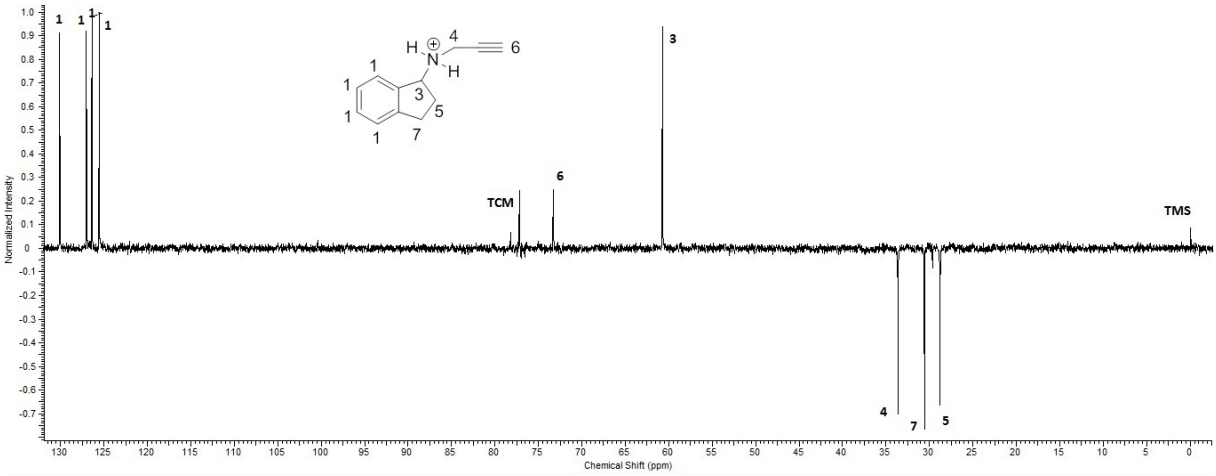


Figure S10b: ^{13}C-NMR spectrum of the isolated product (*R*)-rasagiline (**2a**) in chloroform synthesized by reductive amination applying IR-14.

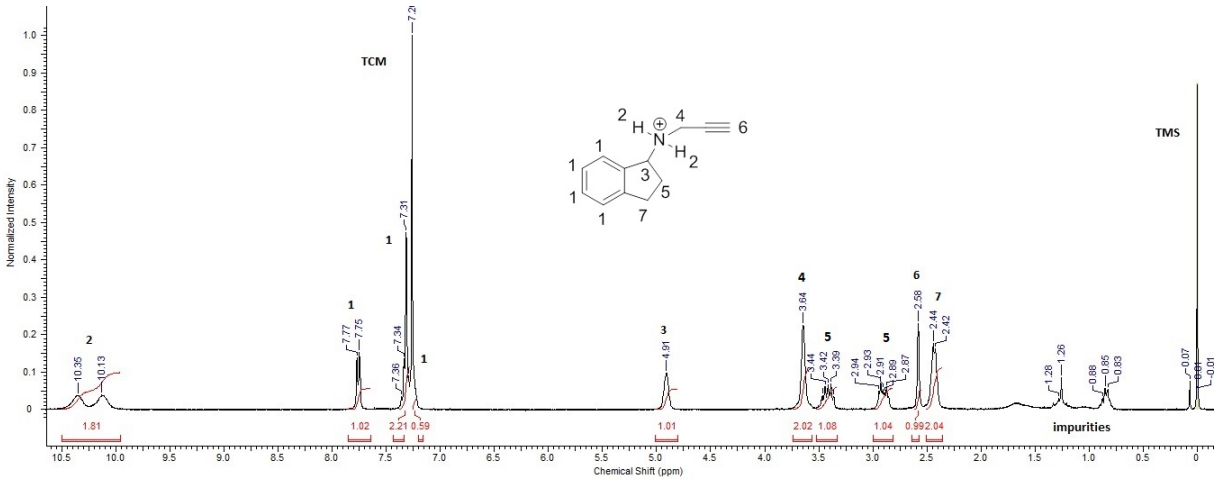


Figure S11a: ^{1}H-NMR spectrum of the isolated product (*S*)-rasagiline (**2a**) in chloroform synthesized by reductive amination applying IR-Sip.

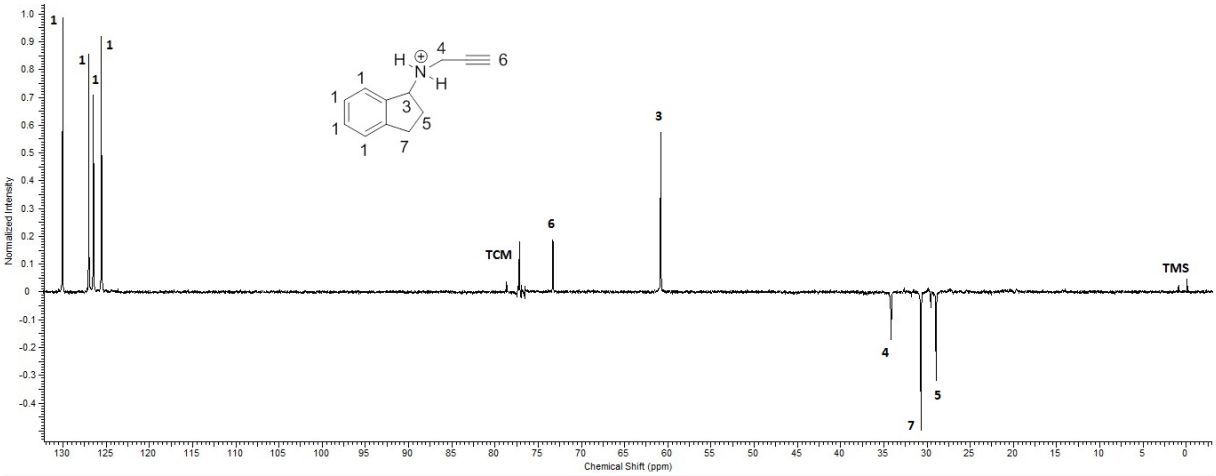


Figure S11b: ^{13}C-NMR spectrum of the isolated product (*S*)-rasagiline (**2a**) in chloroform synthesized by reductive amination applying IR-Sip.

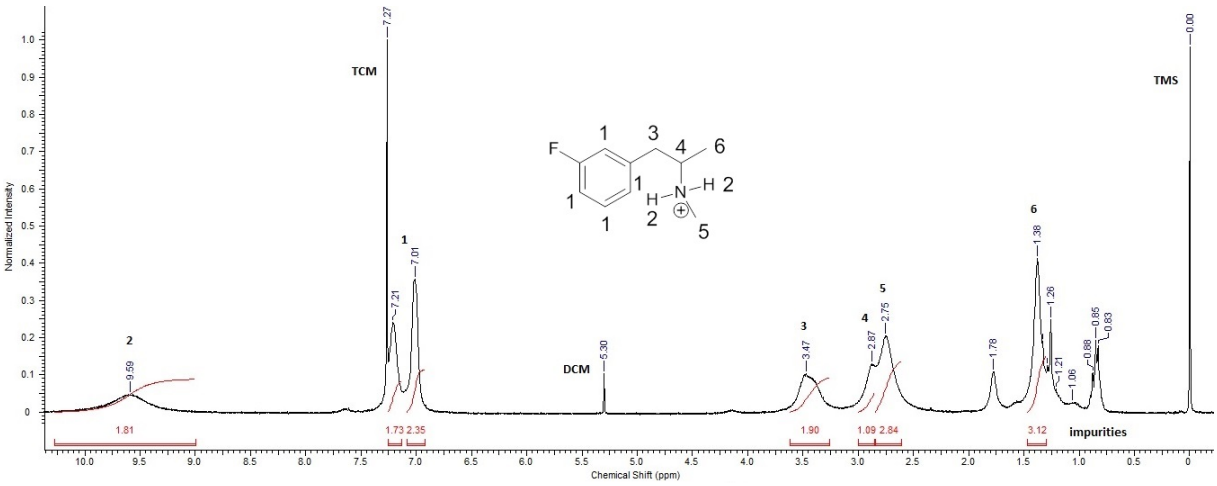


Figure S12a: ^{1}H-NMR spectrum of the isolated product 4-fluoro methamphetamine (**3d**) in chloroform synthesized by reductive amination applying IR-Sip.

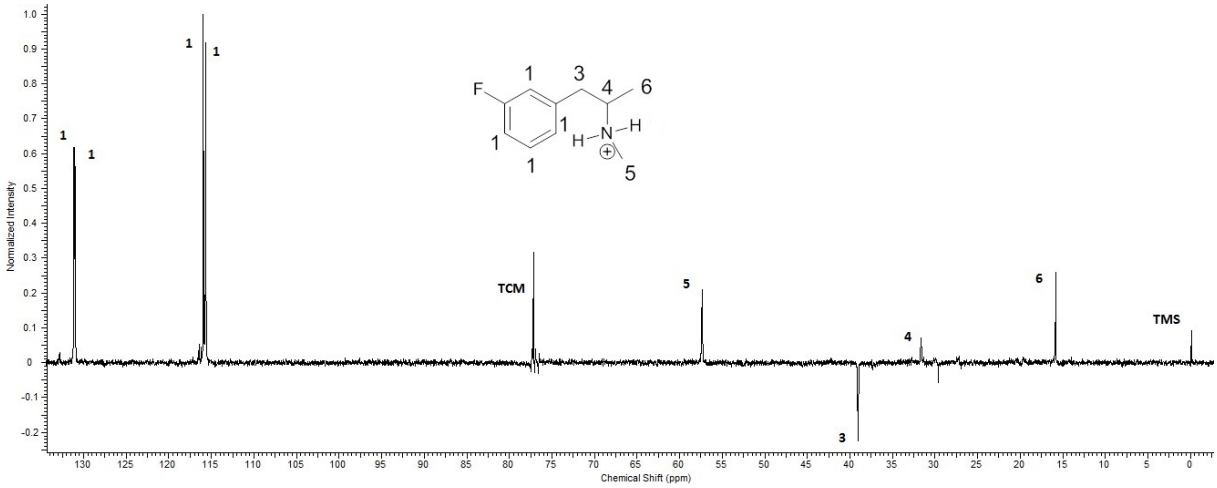


Figure S12b: ^{13}C-NMR spectrum of the isolated product 4-fluoro methamphetamine (**3d**) in chloroform synthesized by reductive amination applying IR-Sip.

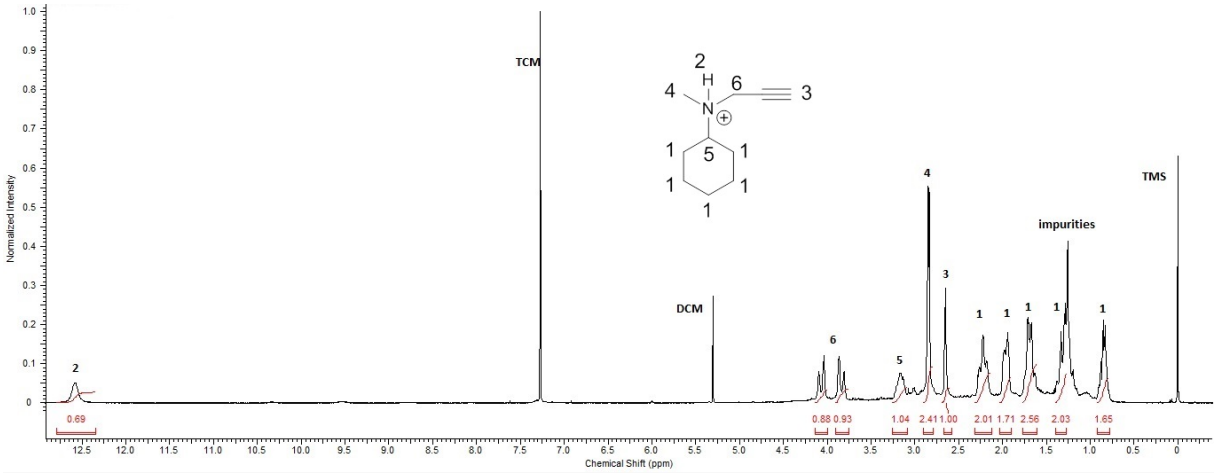


Figure S13a: ^{1}H-NMR spectrum of the isolated product *N*-methyl-*N*-propargyl cyclohexanamine (**1b**) in chloroform synthesized by reductive amination applying IR-14.

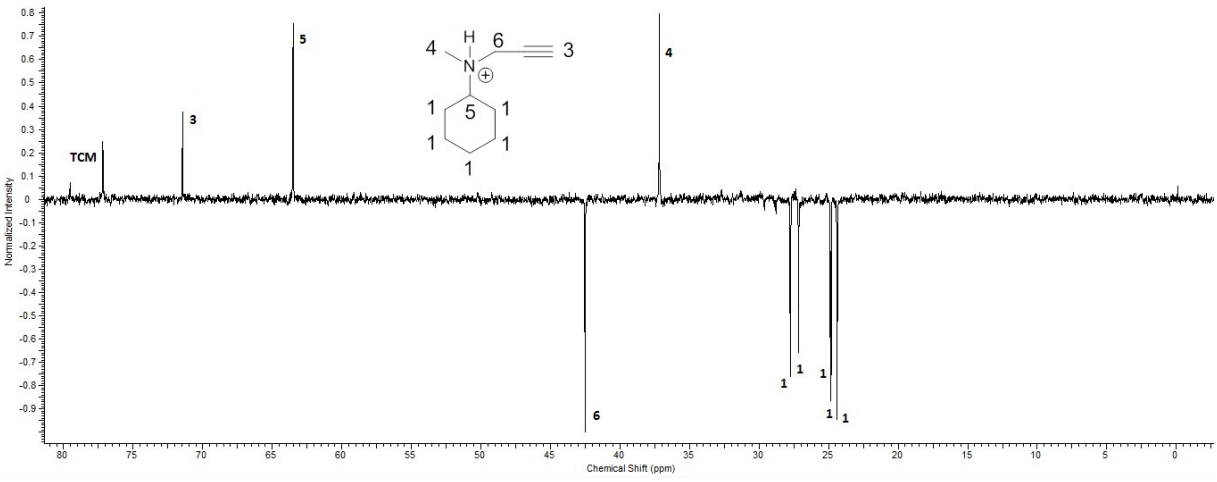


Figure S13b: ^{13}C-NMR spectrum of the isolated product *N*-methyl-N-propargyl cyclohexanamine (**1b**) in chloroform synthesized by reductive amination applying IR-14.

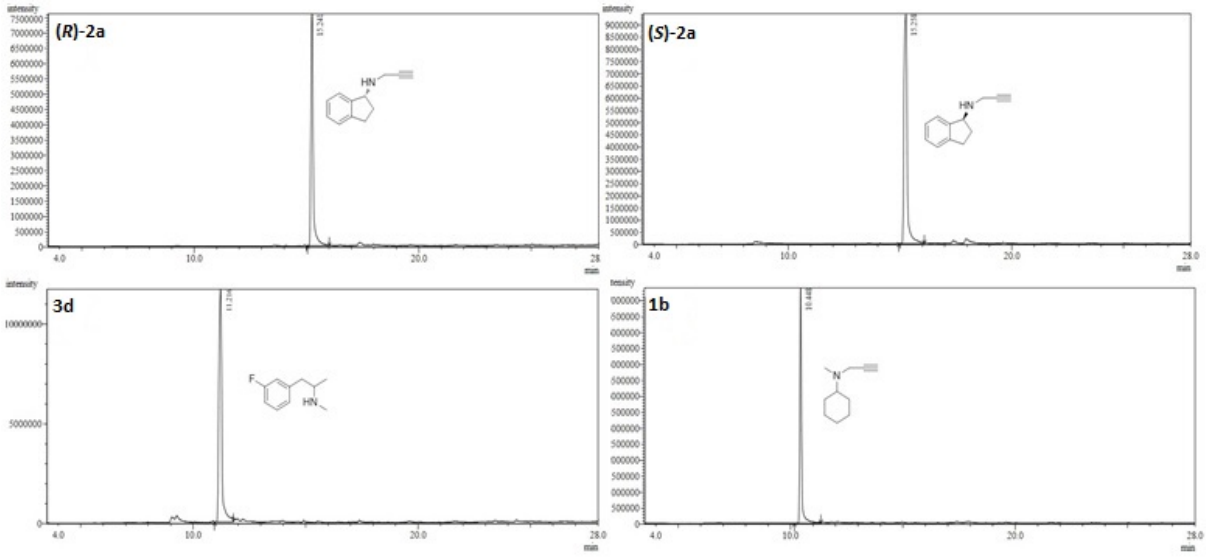


Figure S14: GC chromatograms to investigate the purity of the isolated products ((*R*)-**2a**, (*S*)-**2a**, **3d**, **1b**) (temperature gradient from 60°C to 350°C).

3. Notes and References

3.1. Acknowledges

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3.2. References

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