Supplementary Materials: Immobilized Whole-Cell ω-Transaminase Biocatalysts for Continuous-Flow Kinetic Resolution of Amines

Zsófia Molnár, Emese Farkas, Ágnes Lakó, Balázs Erdélyi, Wolfgang Kroutil, Beáta G. Vértessy, Csaba Paizs [6] and László Poppe

1. Analytical Methods

1.1. Infrared Spectroscopy

Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer and wavenumbers of bands are listed in cm^{-1}.

1.2. Optical Rotation

Optical rotations were measured on Perkin-Elmer 241 polarimeter at the D-line of sodium. The polarimeter was calibrated with measurements of both enantiomers of menthol.

1.3. Gas Chromatography (GC)

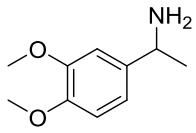
The reaction mixtures from different kinetic resolution reactions were analyzed on an Agilent 4890 GC instrument equipped with flame ionization detector (FID) and a Hydrodex β-6 TBDM column (Macherey-Nagel; 25 m × 0.25 mm × 0.25 μm film thickness of heptakis-(2,3-di-*O*-methyl-6-*O*-t-butyl-dimethylsilyl)-β-cyclodextrin phase). Operation conditions for both instruments FID (250 °C), injector (250 °C), carrier gas H_2 (head pressure 12 psi, split ratio: 1:50).

1.4. NMR

The NMR spectra were recorded in $CDCl_3$ on a Bruker DRX-300 or DRX-500 spectrometer operating at 500 MHz for ^1H and 75 MHz for ^{13}C, and signals are given in ppm on the δ scale.

2. Synthetic methods and Characterization of Products

2.1. Synthesis of racemic 1-(3,4-dimethoxyphenyl)ethan-1-amine rac-1d



rac-**1d**

1-(3,4-Dimethoxyphenyl)ethan-1-one (5 g, 27.7 mmol), ammonium formate (10.5 g, 166.5 mmol), and 10% Pd/C (0.5 g) in methanol (80 mL) was stirred at room temperature. After completion of the reaction the mixture was filtered through Celite® and the solvent was removed by vacuum rotary evaporation. The pH of the residue was adjusted to 1 by aqueous cc. HCl, and and the remaining ketone was removed by extraction with dichloromethane (3 × 40 mL). After removal of the ketone, pH of the aqueous phase was adjusted to 10 by addition of ammonium hydroxide (25%) and the residual amine was extracted with dichloromethane (3 × 40 mL). The unified organic phase was extracted with saturate brine (30 mL) and dried over Na_2SO_4 and concentrated in vacuum to yield the product amine *rac*-**1d** (2.49 g, 50% yield) as yellow oil.

^1H NMR (500 MHz, $CDCl_3$) δ_H: 6.90 (1H, d, Ar*H*), 6.86-6.84 (1H, m, Ar*H*), 6.81-6.80 (1H, m, Ar*H*), 4.07 (1H, q, *J*=6.6 Hz, C*H*), 3.88 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 1.57 (2H, br, NH_2), 1.35 (3H, d, *J*=6.6 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C: 149.17 (CH), 148.02 (CH), 140.63 (CH), 117.77 (CH), 111.26 (CH), 109.20 (CH), 56.12 (OCH_3), 56.03 (OCH_3), 51.20 (CH), 25.99 (CH_3); **IR** (liquid film) υ_{max}: 3359, 3302, 2960, 2934, 2834, 2091, 2021, 1591, 1515, 1462, 1452, 1418, 1369, 1257, 1233, 1138, 109, 1024, 852, 807, 763, 644, 615, 575, 463 cm^{-1}.

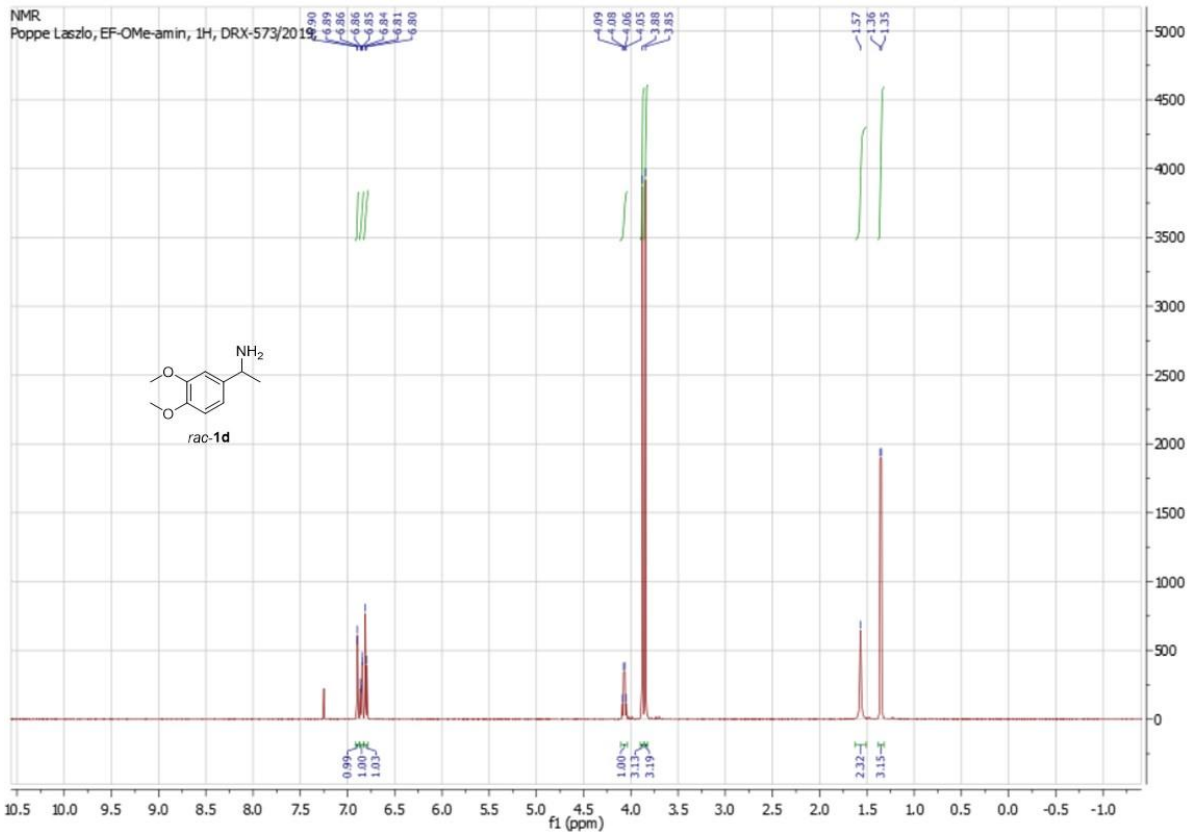


Figure S1. ^{1}H-NMR spectrum of *rac*-**1d.**

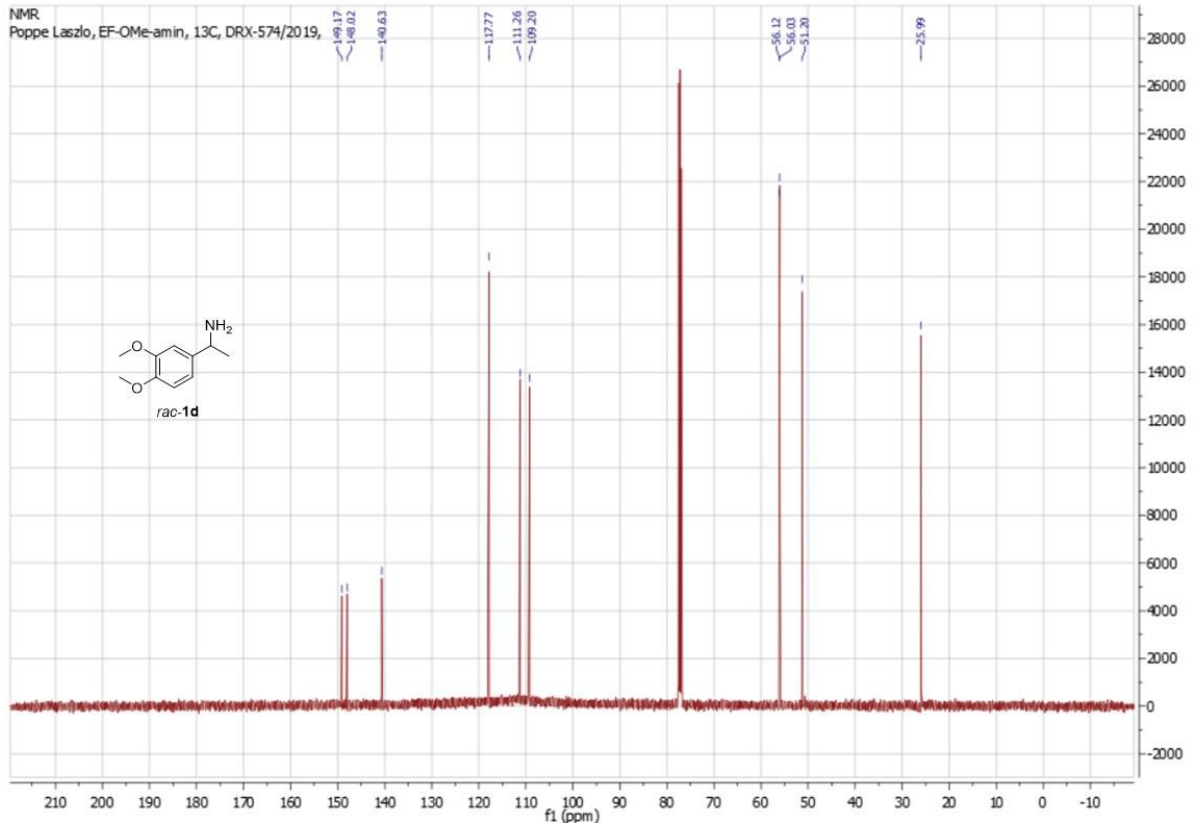


Figure S2. ^{13}C-NMR spectrum of *rac*-**1d.**

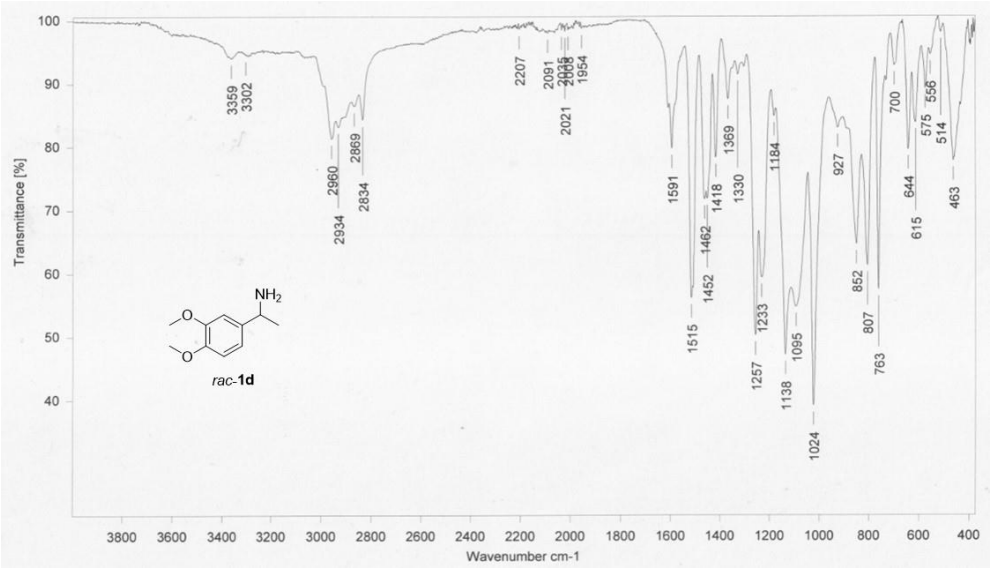


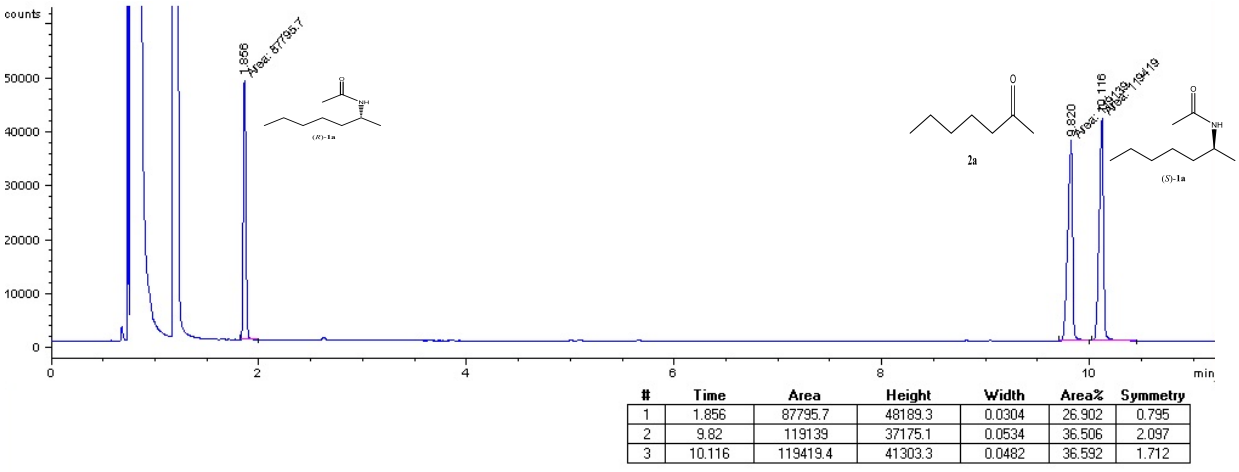
Figure S3. FT-IR spectrum of *rac*-**1d**.

2.2. GC data and Chromatograms of the Investigated Compounds

Table S1. GC data of reference substrates and products.

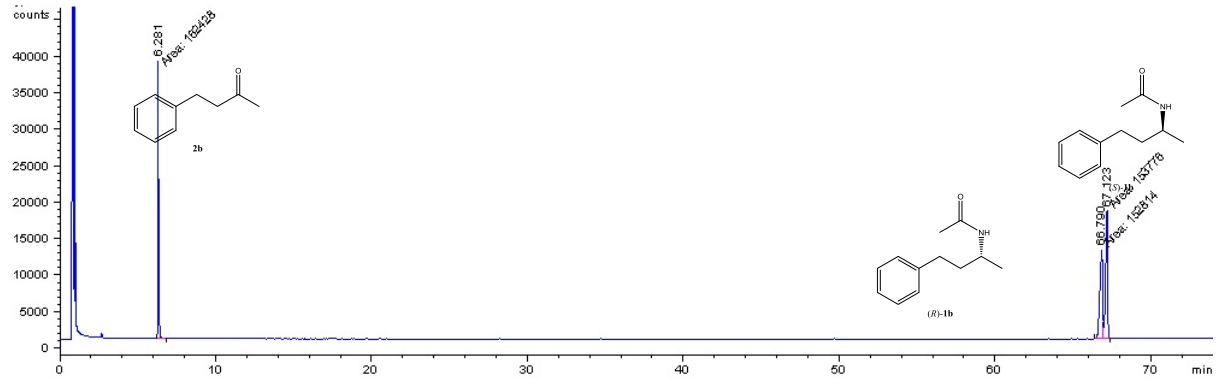
Entry	Compound	Oven Temperature	Retention Time [min]			Ref. Chrom.	Response Factors
			ketone	*Amide* [a]			*rac*-**1** : **2**
1	**1a**	100-160 °C 4 °C/min; 160-180 °C, 20 °C/min; 180 °C, 1 min	1.86	9.82	10.11	FigS4	1.85
2	**1b**	130 °C 60 min; 130-140 °C, 2 °C/min, 140-180 °C, 10 °C/min; 180 °C, 3 min	6.28	66.79	67.12	FigS5	1.20
3	**1c**	100-180 °C, 4 °C/min	4.74	16.15	16.37	FigS6	1.15
4	**1d**	150 °C 40 min; 150-170 °C, 1 °C/min; 170 °C, 15 min	13.11	64.54	65.30	FigS7	1.23

[a] After derivatization to acetamide with acetic anhydride.



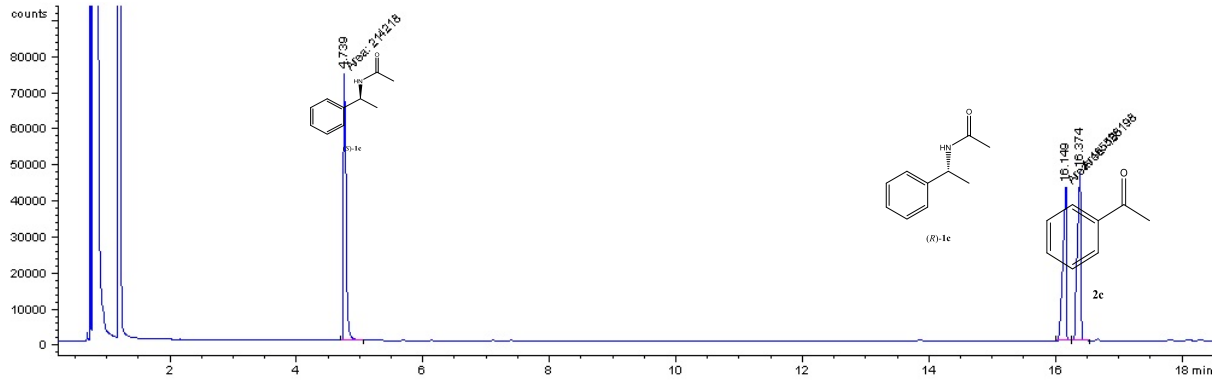
#	Time	Area	Height	Width	Area%	Symmetry
1	1.856	87795.7	48189.3	0.0304	26.902	0.795
2	9.82	119139	37175.1	0.0534	36.506	2.097
3	10.116	119419.4	41303.3	0.0482	36.592	1.712

Figure S4. GC chromatogram of *rac*-**1a** and **2a** (Hydrodex β-6 TBDM; oven program: 100-160 °C, 4 °C/min; 160-180 °C, 20 °C/min; 180 °C, 1 min).



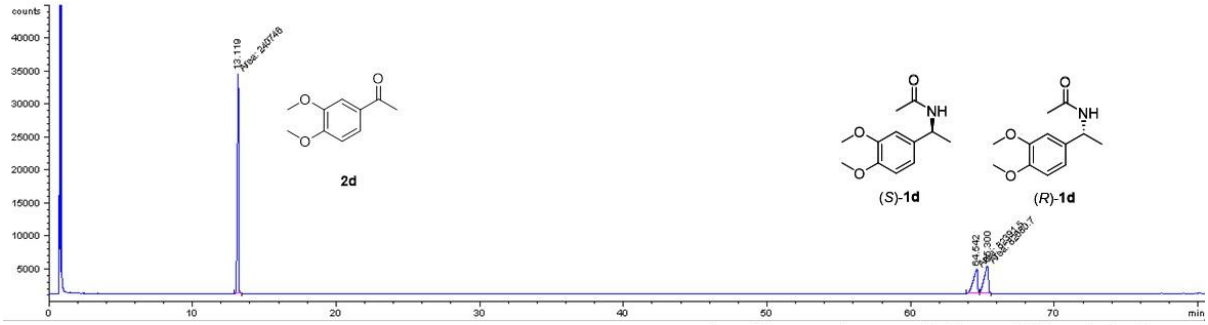
#	Time	Area	Height	Width	Area%	Symmetry
1	6.281	162428.1	38072.3	0.0711	34.631	0.324
2	66.79	152814.2	12151.9	0.2096	32.582	2.124
3	67.123	153776.1	17563.8	0.1459	32.787	1.71

Figure S5. GC chromatogram of *rac*-**1b** and **2b** (Hydrodex β-6 TBDM; oven program: 130 °C, 60 min; 130-140 °C, 2 °C/min; 140-180 °C, 10 °C/min; 180 °C, 3 min).



#	Time	Area	Height	Width	Area%	Symmetry
1	4.739	214218.3	74026.3	0.0482	39.238	0.256
2	16.149	165527.7	42593.6	0.0648	30.320	2.911
3	16.374	166198	46704.8	0.0593	30.442	2.753

Figure S6. GC chromatogram of *rac*-**1c** and **2c** (Hydrodex β-6 TBDM; oven program: 100-180 °C, 4 °C/min).



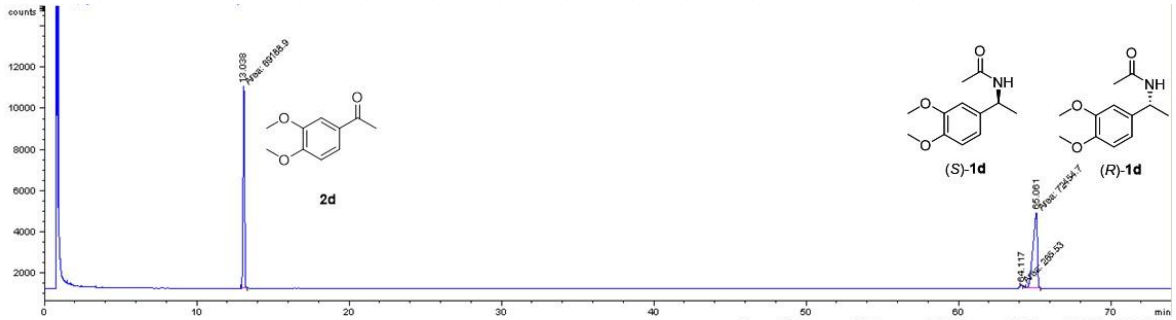
#	Time	Area	Height	Width	Area%	Symmetry
1	13.119	240746	33294.4	0.1205	59.324	1.222
2	64.542	82391.5	3650.5	0.3762	20.303	2.422
3	65.3	82680.7	4154.1	0.3317	20.374	2.704

Figure S7. GC chromatogram of *rac*-**1d** and **2d** (Hydrodex β-6 TBDM; oven program: 150 °C, 40 min; 150-170 °C, 1 °C/min; 170 °C, 15 min.

2.3. Continuous Flow kinetic Resolution at Preparative Scale

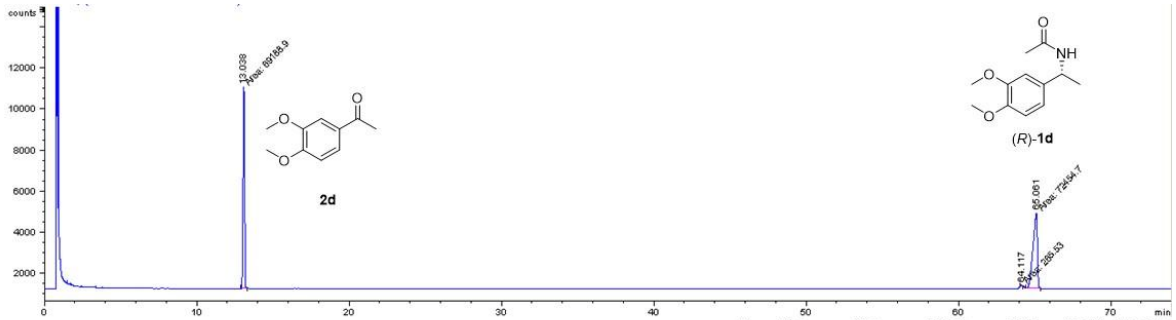
2.3.1. (R)-1-(3,4-Dimethoxyphenyl)ethan-1-amine (R)-**1d**

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 6.91 (1H, m, Ar*H*), 6.87-6.85 (1H, m, Ar*H*), 6.81-6.80 (1H, m, Ar*H*), 4.05 (1H, q, *J*=6.5 Hz, C*H*), 3.89 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 1.78 (2H, br, NH_2), 1.35 (3H, d, *J*=6.5 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C: 149.00 (CH), 147.91 (CH), 117.69 (CH), 111.08 (CH), 109.12 (CH), 55.94 (OCH_3), 55.88 (OCH_3), 51.10 (CH), 25.63 (CH_3); **IR** (liquid film) υ_{max}: 3359, 2960, 2934, 2834, 2091, 2021, 1591, 1515, 1462, 1452, 1418, 1369, 1257, 1233, 1138, 109, 1024, 852, 807, 763, 644, 615, 575, 463 cm^{-1}.



#	Time	Area	Height	Width	Area%	Symmetry
1	13.038	69188.9	9787.4	0.1178	48.756	0.952
2	64.117	265.5	39.4	0.1122	0.187	0.809
3	65.061	72454.7	3663.1	0.3297	51.057	2.714

Figure S8. GC chromatogram of KR from *rac*-**1d** (stationary conditions, Hydrodex β-6 TBDM; oven program: 150 °C, 40 min; 150-170 °C, 1 °C/min; 170 °C, 15 min).



#	Time	Area	Height	Width	Area%	Symmetry
1	13.038	69188.9	9787.4	0.1178	48.756	0.952
2	64.117	265.5	39.4	0.1122	0.187	0.809
3	65.061	72454.7	3663.1	0.3297	51.057	2.714

Figure S9. GC chromatogram of (*R*)-**1d** after working up the KR reaction mixture (Hydrodex β-6 TBDM; oven program: 150 °C, 40 min; 150-170 °C, 1 °C/min; 170 °C, 15 min).

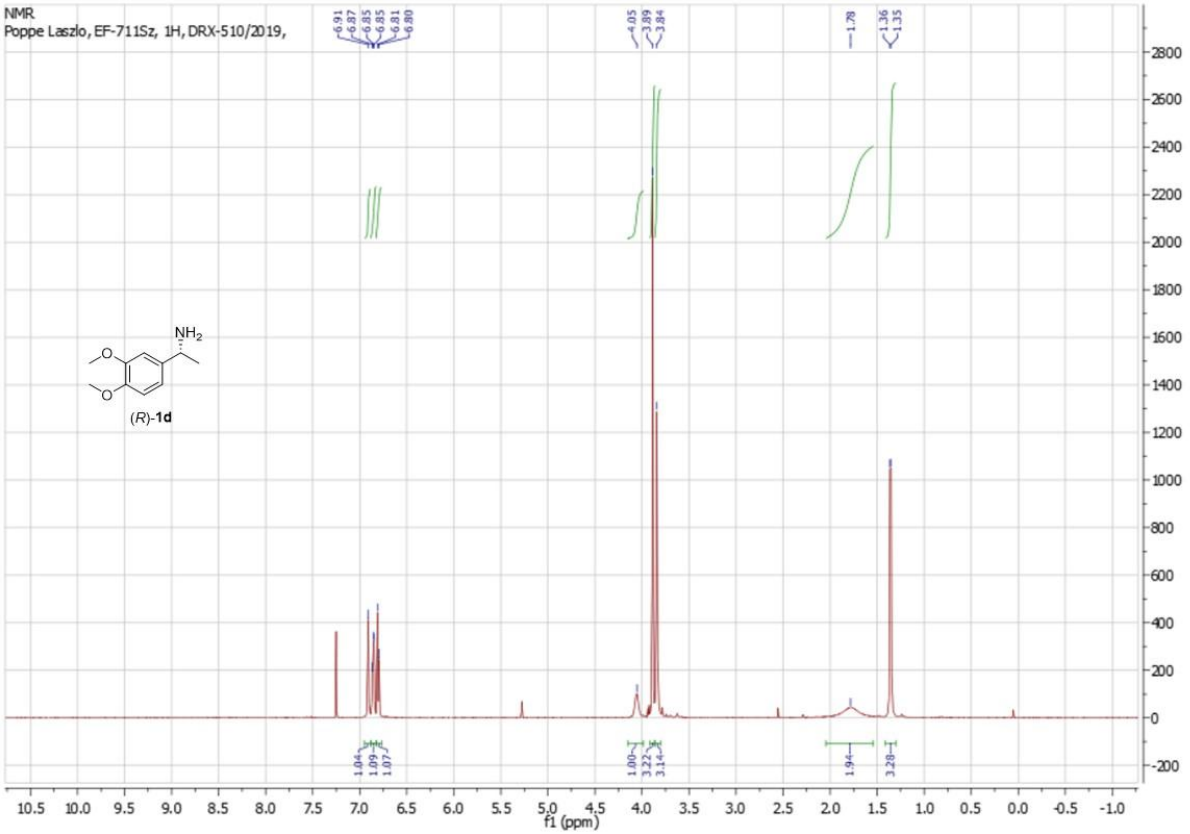


Figure S10. ^{1}H-NMR spectrum of (*R*)-**1d**.

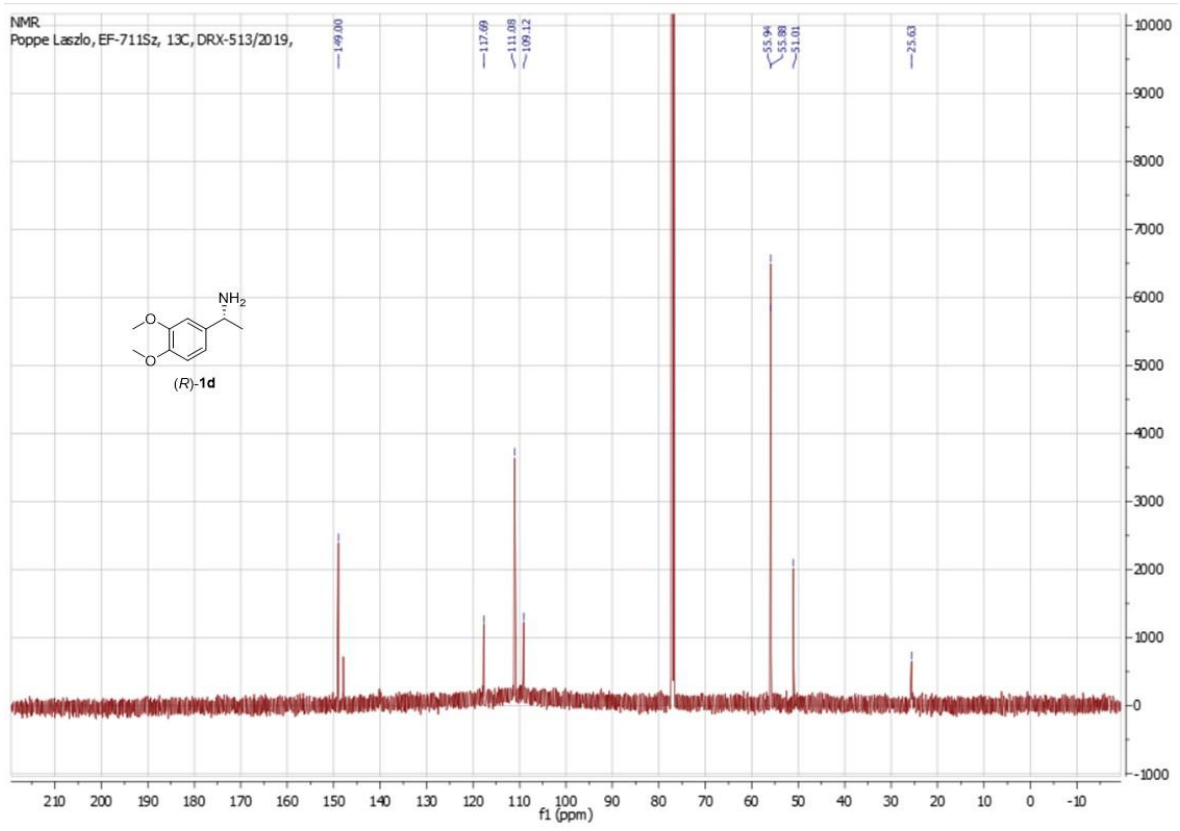


Figure S11. ^{13}C-NMR spectrum of (*R*)-**1d**.

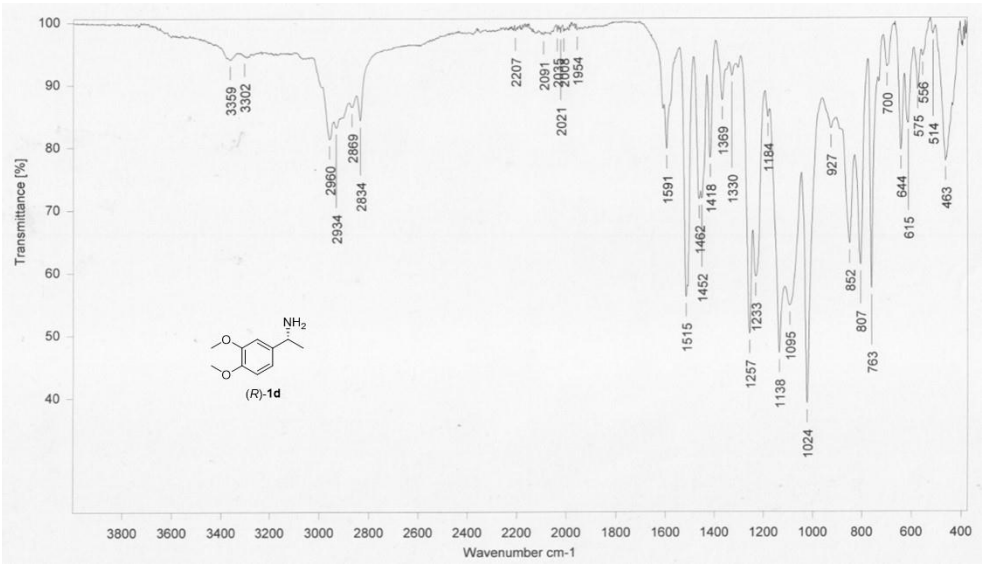
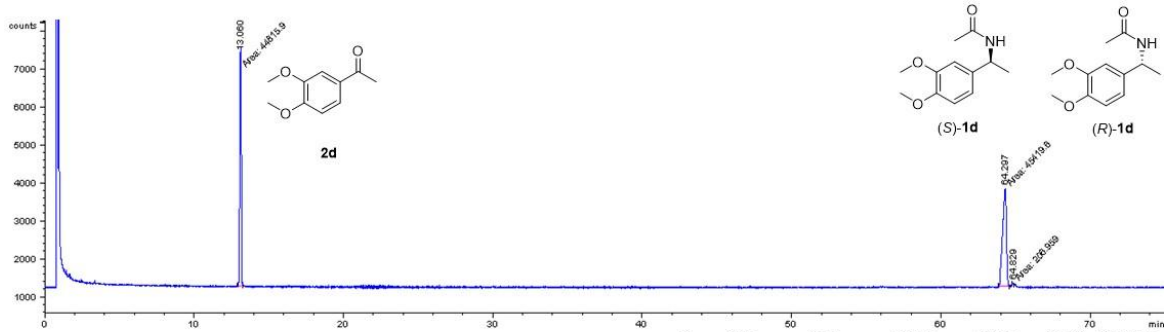


Figure S12. FT-IR spectrum of (*R*)-**1d**.

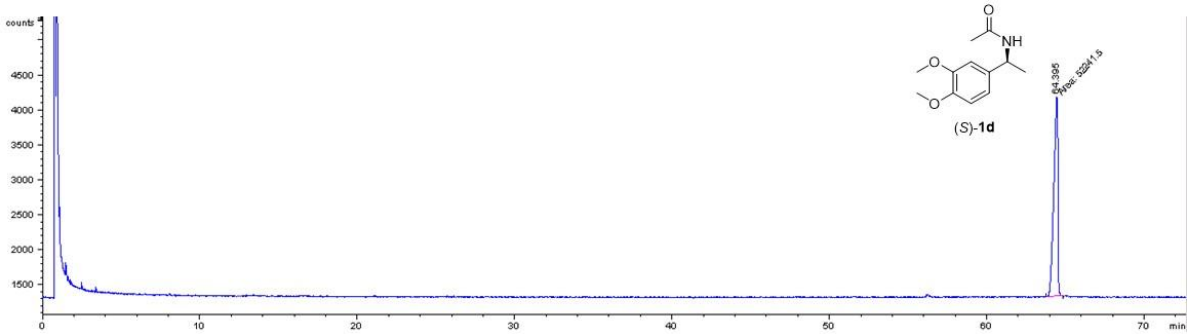
2.3.2. (S)-1-(3,4-Dimethoxyphenyl)ethan-1-amine (S)-**1d**

^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 6.90 (1H, m, Ar*H*), 6.86-6.84 (1H, m, Ar*H*), 6.81-6.80 (1H, m, Ar*H*), 4.07 (1H, q, *J*=6.5 Hz, C*H*), 3.88 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 1.62 (2H, br, NH_2), 1.36 (3H, d, *J*=6.5 Hz, CH_3); **^{13}C NMR** (126 MHz, $CDCl_3$) δ_C: 149.01 (CH), 147.87 (CH), 140.42 (CH), 117.61 (CH), 111.10 (CH), 109.04 (CH), 55.95 (OCH_3), 55.87 (OCH_3), 51.04 (CH), 25.80 (CH_3); **IR** (liquid film) υ_{max}: 3371, 3301, 2997, 2958, 2868, 2834, 1605, 1591, 1515, 1507, 1463, 1451, 1417, 1367, 1305, 1256, 1229, 1138, 1025, 850, 806, 762, 732, 694, 644, 613, 575, 553, 464, 430 cm^{-1}.



#	Time	Area	Height	Width	Area%	Symmetry
1	13.06	44815.9	6280.7	0.1189	49.552	1.021
2	64.297	45419.6	2580	0.2934	50.219	2.085
3	64.829	207	44.1	0.0783	0.229	0.626

Figure S13. GC chromatogram of KR from *rac*-**1d** (stationary conditions, Hydrodex β-6 TBDM; oven program: 150 °C 40 min; 150-170 °C 1 °C/min; 170 °C 15 min).



#	Time	Area	Height	Width	Area%	Symmetry
1	64.395	52241.5	2855	0.305	100.000	2.029

Figure S14. GC chromatogram of (*S*)-**1d** after working up the KR reaction mixture (Hydrodex β-6 TBDM; oven program: 150 °C, 40 min; 150-170 °C, 1 °C/min; 170 °C, 15 min).

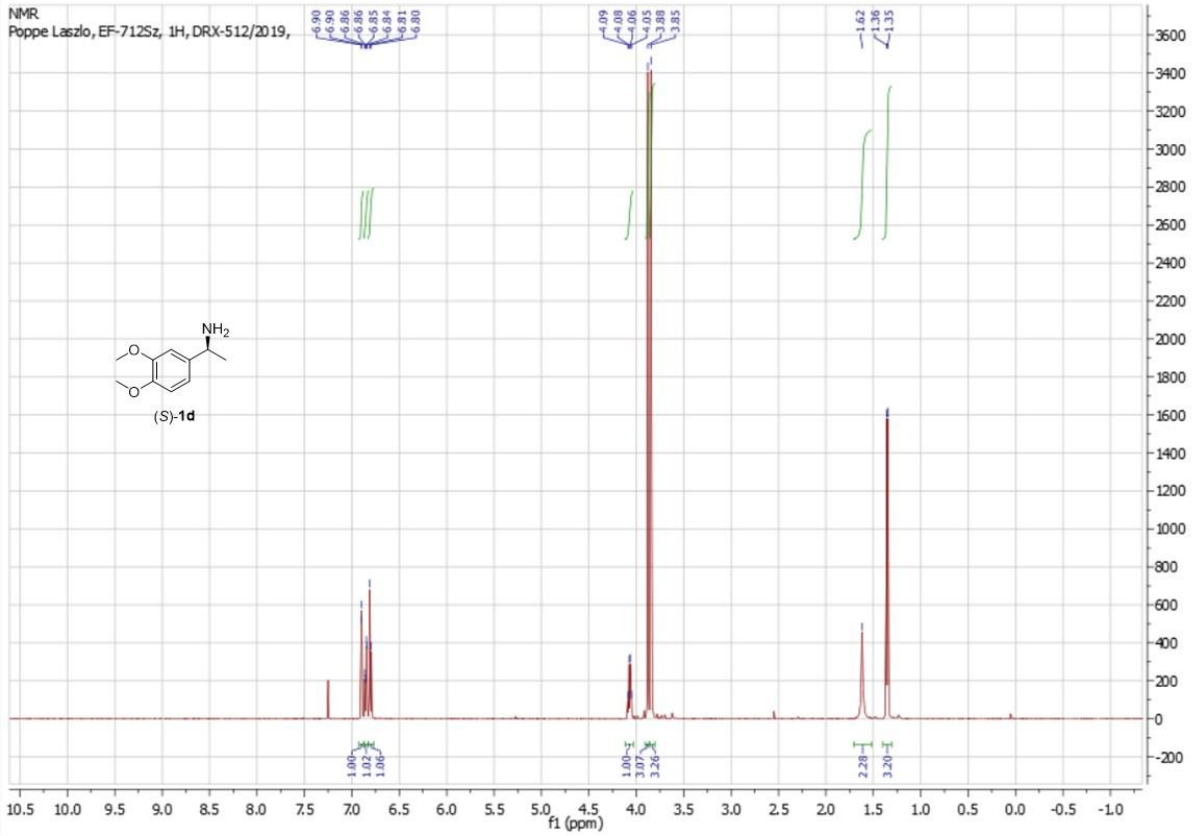


Figure S15. ^{1}H-NMR spectrum of (*R*)-**1d**.

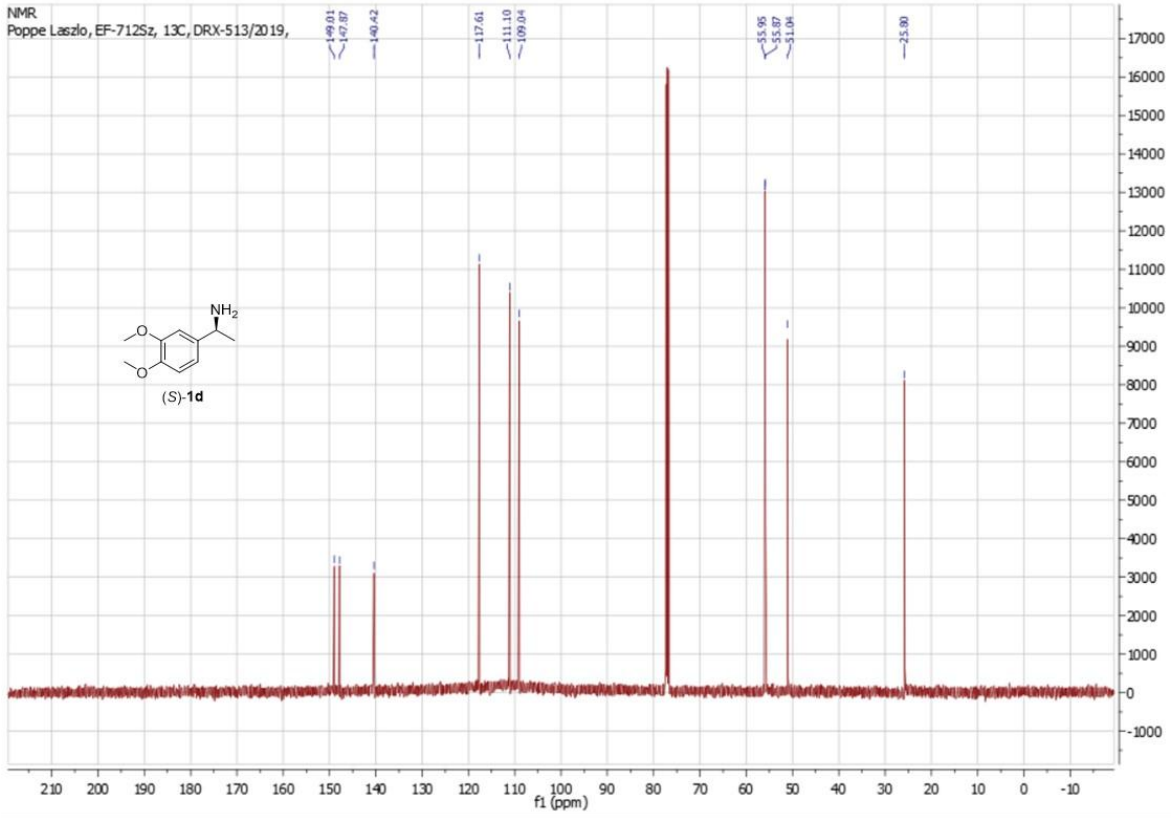


Figure S16. ^{13}C-NMR spectrum of (*R*)-**1d**.

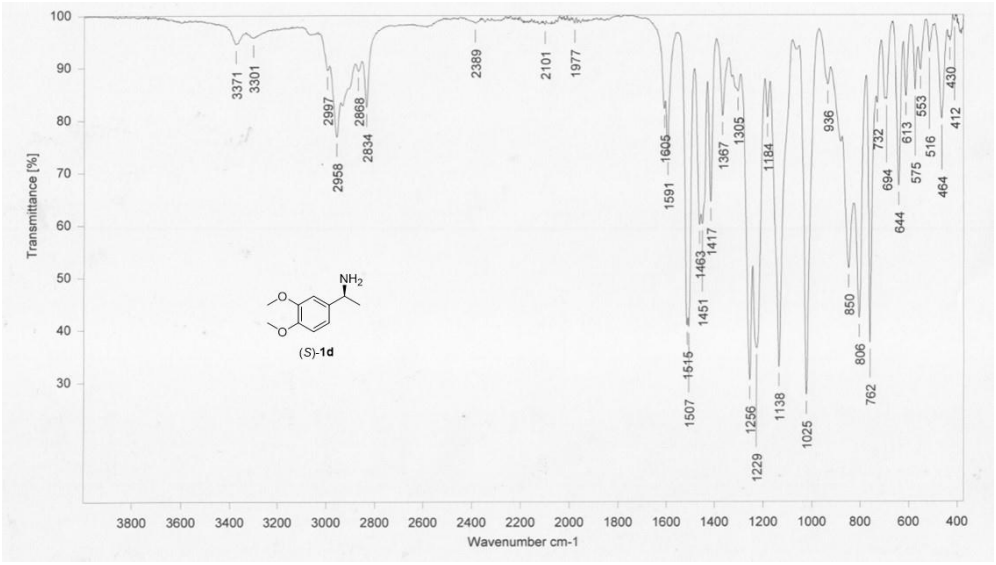
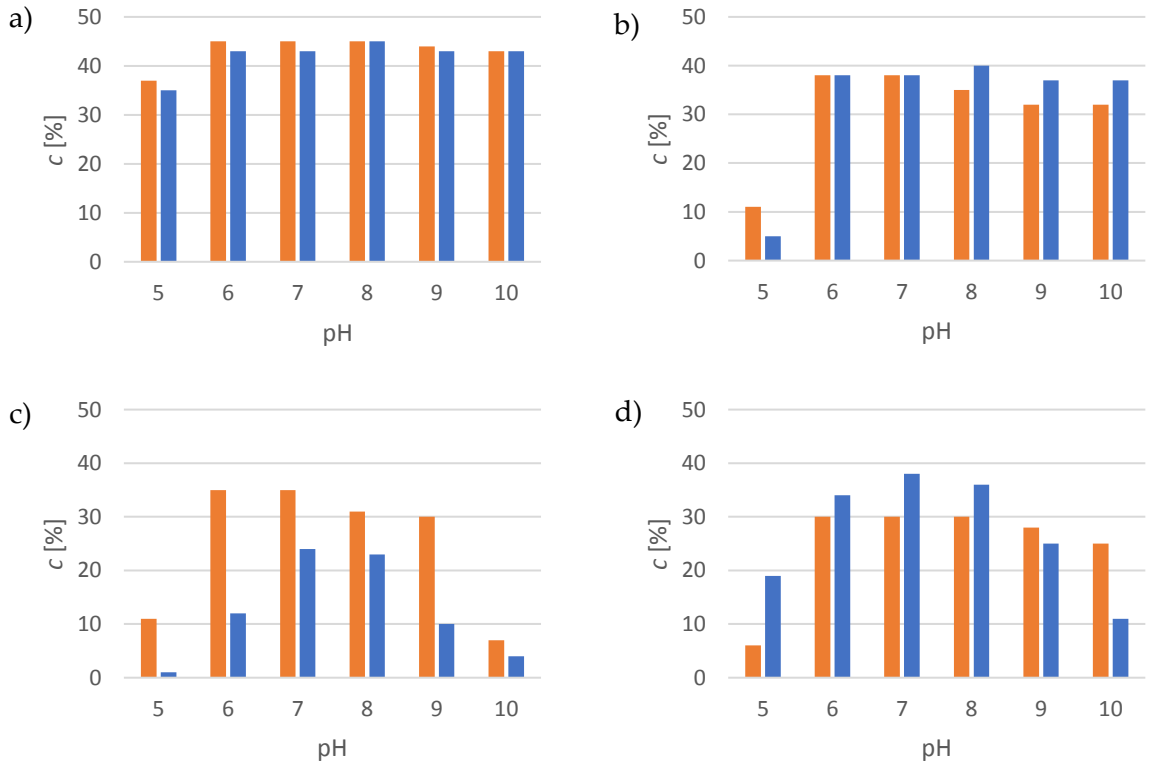


Figure S17. FT-IR spectrum of (*S*)-**1d**.

3. Efficiency of Immobilization and pH Dependence of the TA Biocatalysts

The immobilized TA biocatalyst (50 mg) or the wet TA-containing *E. coli* cells (50 mg wet cell mass) were suspended in buffer set to the corresponding pH (2 mL, 100 mM; the solutions with pH ranging from 4 to 7 were buffered with sodium citrate, the solutions with pH ranging from 8 to 10 were buffered with sodium borate) containing the racemic amine (*rac*-**1c**, 30 mM), sodium pyruvate (22.5 mM) and pyridoxal-5′-phosphate monohydrate (PLP, 0.3 mM) in 4 mL vials. The reaction mixtures were shaken on an orbital shaker (600 rpm) at 30 °C for 24 h.



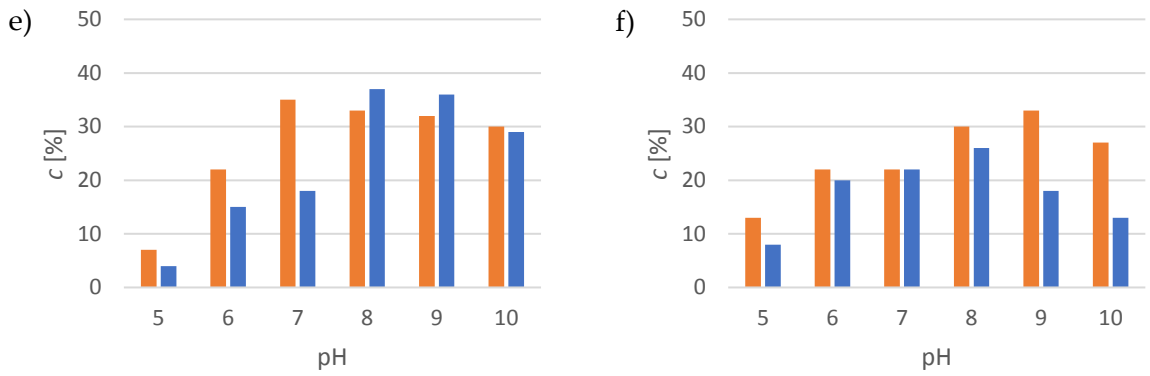


Figure S18. pH dependence of the transaminase containing wet *E. coli* cells (■) and the immobilized TA biocatalysts (■); a) *ArS*-TA; b) *AtR*-TA; c) *CvS*-TA$_m$; d) *ArR*-TA; e) *VfS*-TA; f) *ArR*-TA$_m$.

The cell content of the applied biocatalyst can be evaluated based on the wet cell mass before immobilization and the dry mass of the prepared biocatalyst, by taking into account the ~100% cell-immobilization yield determined in the previous work of our research group. From 1 g of wet cell approx. ~0.9 g of dry TA biocatalyst can be produced. Activity yield could be determined by comparison of the activity data with the wet cells vs. with the immobilized TA biocatalysts. Consequently, at a wide range of pH values, activity yields in the range of 80 – 105% could be observed for the six immobilized TA biocatalysts.