Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.

Lipase on carbon nanotube - An active, selective, stable and easy to optimize nanobiocatalyst for kinetic resolutions

Cristian Andrei Gal, Laura Edit Barabás, Judith-Hajnal Bartha Vári, Mădălina Elena Moisă, Diana Balogh-Weiser, László Csaba Bencze, László Poppe, Csaba Paizs and Monica Ioana Toșa

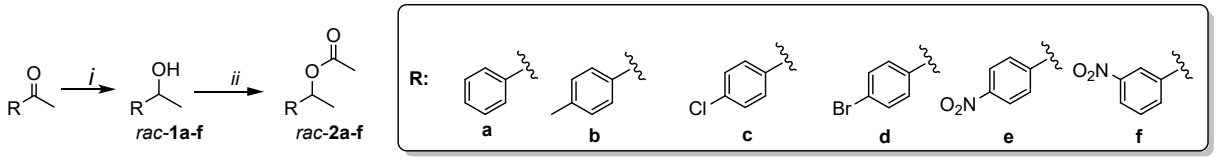
1. Chemical synthesis of *rac*- 1-2a-f

1.1. Synthesis of racemic alcohols *rac*-1a-f

Details of preparation were already reported for *rac*-**1a,c,d** [1], *rac*-**1b** [2], *rac*-**1e** [3], *rac*-**1f** [4]. According to the general method, the corresponding ketone (2 g) was dissolved in methanol (50 mL), and $NaBH_4$ (2 equiv.) was added under continuous stirring on cold water bath. After completion of the reaction (checked by TLC with CH_2Cl_2 as eluent), the mixture was diluted with water (20 mL), acidified with 10% HCl solution (pH=4, 10 mL), and stirred for another 10 minutes (**Scheme S1**). The pH was set to 8 with a 10% Na_2CO_3 solution. The aqueous solution was extracted with dichloromethane (3 × 20 mL). The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified on column chromatography using CH_2Cl_2 as eluent, to afford the corresponding pure alcohols *rac*-**1a-f**. The structure of all compounds was confirmed through ^{1}H- and ^{13}C- NMR spectra.

1.2. Chemical acylation of the racemic alcohols *rac*-1a–f

Acetyl chloride (2 equiv.) and DMAP (2.2 equiv.) were added into the solution of the racemic alcohols *rac*-**1a-f** (0.2 g) in CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 hours until completion (checked by TLC with CH_2Cl_2 as eluent). The reaction mixture was washed with HCl solution 3% (2 ×5 mL), water (5 mL), and a solution of Na_2CO_3 1M (5 mL) (**Scheme S1**). The organic layer was dried over anhydrous sodium sulfate and the solvent was distilled off by rotatory evaporation. The crude product was purified by chromatography using CH_2Cl_2 as eluent to give the pure acetates *rac*-**2a–f**. The structure of all compounds was confirmed through ^{1}H- and ^{13}C- NMR spectra.



Scheme S1. Chemical synthesis of the racemic arylethan-1-ols and their acetates *rac*-**1,2a-f**

2. Chromatographic separation of *rac*- 1-2a-f

The base line chromatographic separation of the enantiomers was established (Table S1). GC analysis was performed on an Agilent 7890A GC gas chromatograph equipped with a flame ionization detector using an Agilent 7890A gas chromatograph equipped with a flame ionization detector using a 30 m x 0.25 mm Astec CHIRALDEX B-DM capillary column (30m × 0.32mm × 0.12 μm). The analysis conditions were the following: 1 μL injection volume, 100:1 split ratio, carrier gas N_2; head pressure: 60 psi, injector: 250 °C; FID detector: 250 °C. and heating at 250 °C and a DB-WAX chiral capillary column (30m × 0.32mm × 0.5 μm).

High Performance Liquid Chromatography (HPLC) analyses were conducted with an Agilent 1200 instrument equipped with a quaternary pump operated at 1 mL/min. flow rate, column thermostat (25 °C), and with a UV-Vis DAD (280 nm wavelength). For the chiral separation of *rac*-**1,2e,f** a Chiralpack ASH column (250 mm × 4.6 mm × 5 μm) was used.

Table S1 Chromatographic chiral separation of the ethanols *rac*-**1a-f** and their acetates *rac*-**2a-f**

Compound	R	Method	HPLC/GC Column	Temperature (°C)/ mobile phase (H:IPA, v:v)	Retention time (min)
rac-**2a** (*R*/*S*)	phenyl	GC	β-BM	120	5.5/5.9
rac-**1a** (*S*/*R*)		GC	β-BM	120	7.4/7.6
rac-**2b** (*R*/*S*)	4-methyl-phenyl	GC	β-BM	130	6.1/6.4
rac-**1b** (*R*/*S*)		GC	β-BM	130	6.5/6.8
rac-**2c** (*S*/*R*)	4-chloro-phenyl	GC	β-BM	120-160 (2.6 grd/min)	9.4/9.8
rac-**1c** (*R*/*S*)		GC	β-BM	120-160 (2.6 grd/min)	11.3/11.6
rac-**2d** (*S*/*R*)	4-bromo-phenyl	GC	β-BM	120	24.9/26.6
rac-**1d** (*S*/*R*)		GC	β-BM	120	28.3/31.3
rac-**2e** (*S*/*R*)	4-nitro-phenyl	HPLC	ASH	87:13	8.8/12.4
rac-**1e** (*S*/*R*)		HPLC	ASH	87:13	15.4/19.8
rac-**2f** (*S*/*R*)	3-nitro-phenyl	HPLC	ASH	90:10	6.6/11.8
rac-**1f** (*S*/*R*)		HPLC	ASH	90:10	9.5/10.1

3. Covalent binding of CaL-B to $SwCNT_{COOH}$

$SwCNT_{COOH}$ (20 mg) was activated with *N,N'*-carbonyldiimidazole (CDI, 32.4 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) under shaking at 1350 rpm and room temperature, overnight, with occasional sonication, to avoid bundled SwCNT formation. The sample was filtered on membrane filter and then washed with CH_2Cl_2 (3×5 mL). Into the suspension of the CDI-activated $SwCNT_{COOH}$ (approx. 20 mg) in distilled water (5 mL), 1,3-propanediamine, 1,6-hexanediamine and 1,8-octanediamine (0.12 mmol) was added and the reaction mixture was shaken (at 1350 rpm and room temperature, overnight), with occasional sonication to avoid bundled SwCNT formation. The reaction mixture was filtered on membrane filter, washed with distilled water (3×5 mL) and then dried. In the solution of glycerol diglycidyl ether (GDE, 100 μL) in CH_2Cl_2 (5 mL), diamine-coupled $SwCNT_{COOH}$ (20 mg) was suspended and the reaction mixture was shaken (at 1350 rpm and room temperature, overnight), with occasional sonication to avoid bundled SwCNT formation. The mixture was filtered on membrane filter and then washed with CH_2Cl_2 (3×5 mL). In the solution of CaL-B (5 mg, 10 mg, 20 mg, 40 mg and 100 mg respectively) and Tween-80 (6.5 μL)

in PBS buffer (20 mM Na_2HPO_4, 150 mM NaCl, pH 7, 6 mL) the bisepoxide-activated $SwCNT_{COOH}$ (20 mg) was added and the mixture was shaken at room temperature at 1350 rpm, overnight. The resulted biocatalyst ($SwCNT_{COOH}$-C_n-NH_2-GDE-CaL-B, n= 3, 6 or 8) was filtered on a membrane filter and several time resuspended in water (10 mL) and sonicated until no protein trace was detected in the filtrate. After that, immobilized enzymes were freeze-dried and used in enzymatic transesterifications. The amount of the immobilized CaL-B on the bisepoxide-activated $SwCNT_{COOH}$ was determined as the difference between the total mass of the enzyme in the solution before immobilization and in the unified filtrates after immobilization.

The enzyme preparations were characterized by high immobilization yields (> 99% of the enzyme bound to the support; enzyme loading: 0.16/0.33/0.5/0.66/0.83 mg protein/mg immobilized preparation).

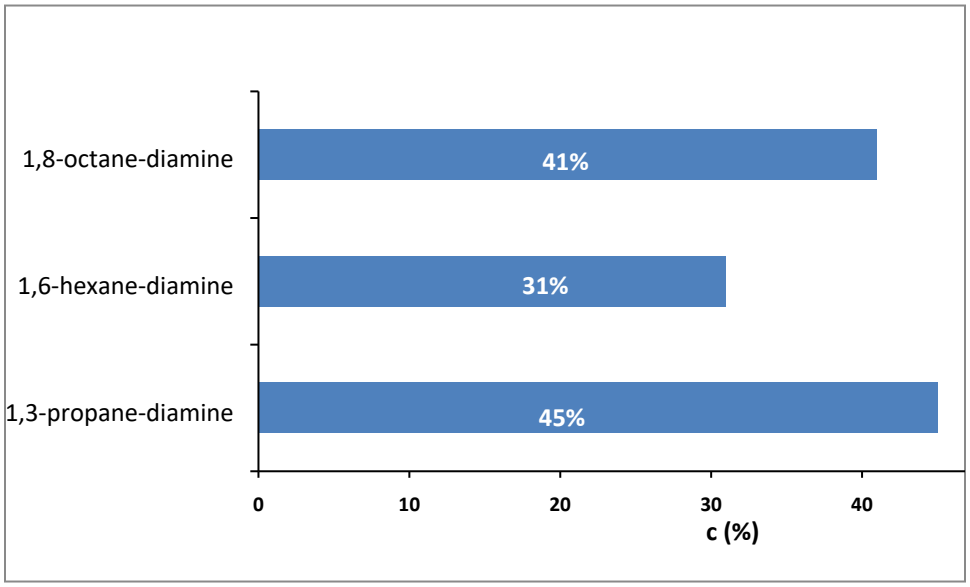


Figure S1. The linker length effect on biocatalyst performance (free enzyme-support *ratio* 1:2, 0.75 equiv. vinyl acetate, *n*-hexane, 30°C, after 2 hours)

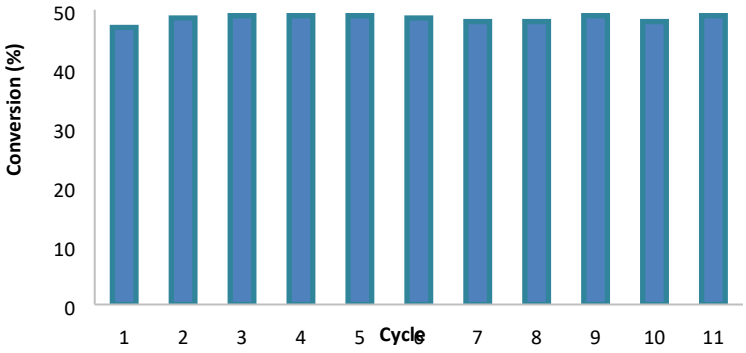


Figure S2. Reusability of the immobilized enzyme preparation (2 mg/mL of *rac*-**1a**) with vinyl acetate (0.75 equiv.) in *n*-hexane, after 1h reaction time.

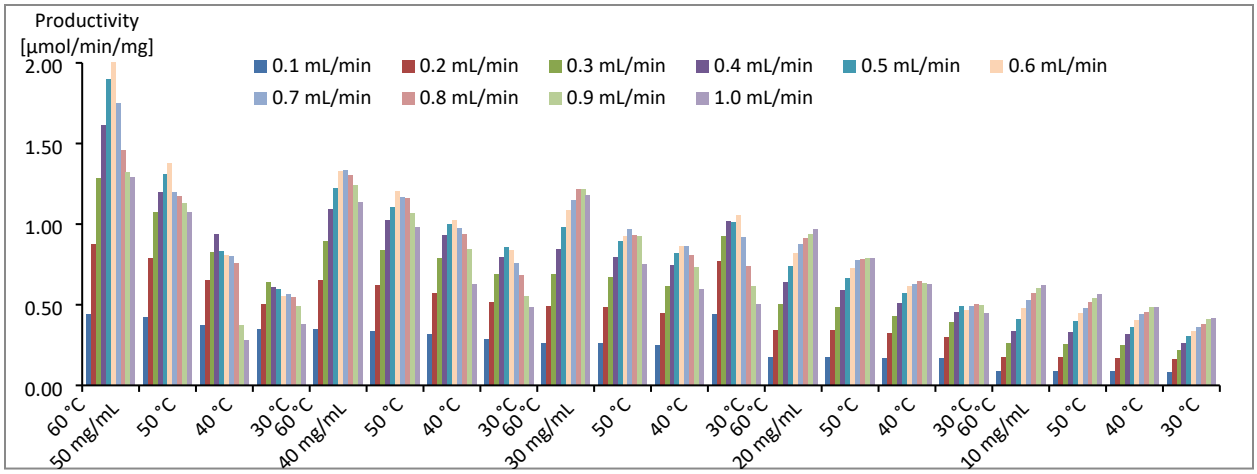


Figure S3. The productivity of flow system as function of temperature, substrate concentration and flow rate

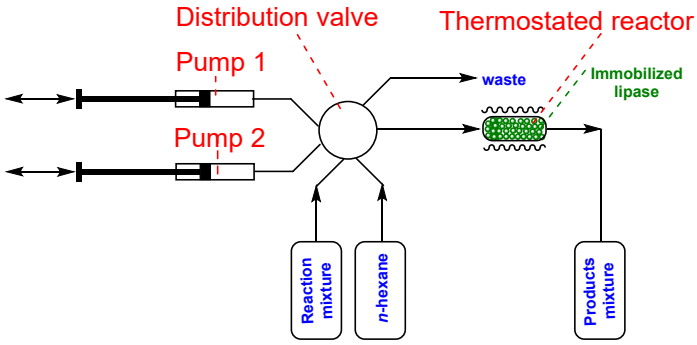


Figure S4. The scheme of the used apparatus in the flow experiments

References

[1] S. Kamble, S. More and C. Rode, *New Journal of Chemistry* 2016, **40**, 10240-10245.

[2] J. V. Comasseto, L. H. Andrade, Á. T. Omori, L. F. Assis, A. L. Meleiro Porto, *Journal of Molecular Catalysis B: Enzymatic* 2004, **29 (1–6)**, 55-61.

[3] L. A. De Almeida, T. H. Marcondes, C. D. F. Milagre, H. M. S. Milagre, *ChemCatChem* 2020, **12,** 2849 - 2858.

[4] B. A. Barros-Filho, M. C. F. de Oliveira, T. L.G. Lemos, M. C. de Mattos, G. de Gonzalo, V. Gotor-Fernández, V. Gotor, *Tetrahedron: Asymmetry* 2009, **20(9)**, 1057-1061.