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

A robust and efficient lipase based nanobiocatalyst for phenothiazinyl-ethanols resolution

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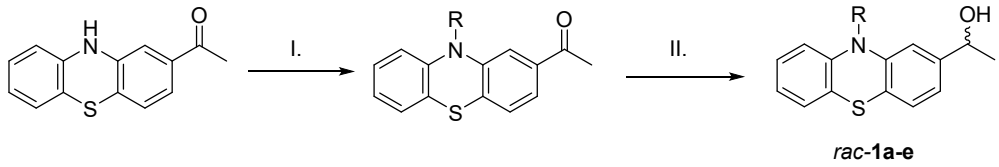
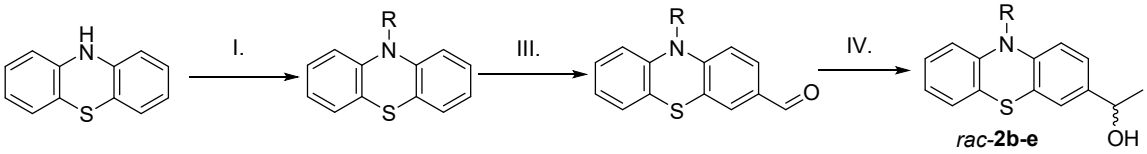
Chemical synthesis of the racemic substrates *rac*-1a-e and *rac*-2b-e

The synthesis of the investigated racemic substrates *rac*-**1a-e** and *rac*-**2b-e** was performed using known methods[1-3]. The racemic *N*-alkyl-phenothiazinyl-2-ethanols were obtained from the corresponding ketones by chemical reduction with sodium borohydride ($NaBH_4$) in methanol, while *N*-alkyl-phenothiazinyl-3-ethanols were obtained from the corresponding aldehydes by Grignard reaction.



rac-**1a-e**



rac-**2b-e**

R: a = H, b = Et, c = *n*-Pr, d= *n*-Bu, e = *n*-Hex

I. DMF, NaH (1.1 Eq.), -5-0 °C, 10 min, R-I/R-Br (1.25 Eq.), 50°C;
II. $NaBH_4$ (1.1 Eq.), MeOH anh., r.t;
III. DMF, $POCl_3$ -5-0 °C, 30 min, reflux, 90 °C
IV. Et_2O,argon, MeMgI (2 Eq.), over night, r.t.

Chemical synthesis of *rac*-1a-e

Into the solution of 2-acetyl-phenothiazin (1g, 4.15 mmol) in dimethylformamide (DMF, 20 mL) sodium hydride (NaH, 1.1 Eq.) was added in small portions at temperature between -5 and 0°C and stirred for 10 min. After 10 min, the alkyl iodide/akyl bromide (1.25 Eq.) was added and the mixture was stirred at 50 °C until completing the reaction (checked by TLC). The solvent was evaporated under vacuum and the resulted mixture was dissolved in dichloromethane (DCM), dried on anhydrous sodium sulfate, filtrated and concentrated. The crude obtained ketones were chemically reduced using 1.1 Eq. of $NaBH_4$ in anhydrous methanol at room temperature. For

work-up, 1 mL of HCl 10% was added dropwise. The solvent was evaporated, and the products were extracted using a mixture of H_2O:DCM (1:2, *v*/*v*). The organic phase was dried on anhydrous sodium sulfate, filtrated, concentrated and the corresponding *N*-alkyl-phenothiazinyl-2-ethanols (*rac*-**1a-e**) were purified by chromatography column on silica gel using DCM as eluent.

Chemical synthesis of *rac*-2b-e

Into the solution of phenothiazine (1g, 4.15 mmol) in DMF (20 mL) sodium hydride (NaH, 1.1 Eq.) was added in small portions at temperature between -5 and 0°C under stirring. After 10 min, the alkyl iodide/akyl bromide (1.25 Eq.) was added and the reaction was perfected under stirring at 50 °C (checked by TLC). The solvent (DMF) was removed under vacuum, the crude products (*N*-alkyl-phenothiazines) were dissolved in dichloromethane (DCM), dried on anhydrous sodium sulfate (Na_2SO_4 anh.), filtrated and concentrated.
In a round bottom flask containing DMF (30 mL) phosphorus oxychloride ($POCl_3$, 10 mL) was added dropwise at temperature between -5 and 0°C under stirring. After 30 min the solution of *N*-alkyl-phenothiazine in hot DMF (5 mL) was added and left for 15 min under stirring at temperature between -5 and 0°C, then at 90 °C for 2 h. The mixture was poured into 600 g of ice and manually stirred; sodium acetate was added to adjust the pH to 6 and left overnight. The products were extracted in toluene until the organic phase remain clear, the organic phase was dried on Na_2SO_4, filtrated, concentrated and purified on silica gel with toluene as eluent, resulting in the 3-formyl derivatives, which were further purified by column chromatography on silica gel (with DCM as eluent) and used as reagents for the synthesis of *N*-alkyl-phenothiazinyl-3-ethanols (*rac*-**2b-e**) by Grignard reaction.
For the Grignard reaction, magnesium (89 mg, 3.7 mmol) was put in round bottom flask with one crystal of I_2 and heated for activation. 5 mL of diethyl ether were added under Argon, followed by methyl iodide (1.1 Eq.) at 45 °C. The 3-formyl derivatives previously dissolved in Et_2O, were poured over resulted solution through a syringe with a long needle. The reaction was perfected under stirring overnight at 45 °C. For work-up, the reaction was quenched by adding a mixture of H_2O and acetic acid (15 mL, 2:1, *v*/*v*). The separated aqueous phase was washed with Et_2O (2 × 10 mL). The combined organic phases were dried on Na_2SO_4, filtered, concentrated and *N*-alkyl-phenothiazinyl-3-ethanols (*rac*-**2b-e**) were purified on silica gel using a mix of *n*-hexan:ethyl acetate (6:4, *v*/*v*) as eluent.

The yields for the *N*-alkyl-phenothiazinyl-ethanols preparation are presented in Table S1.
The structures of seven *N*-alkyl-phenothiazinyl-ethanols (*rac*-**1a-d** and *rac*-**2b-d**) were already confirmed in previous works, alongside with the chromatographic separation method[1-3]. The structures of *N*-n-hexyl derivatives *rac*-**1e** and *rac*-**2e**, substrates that were not previously synthetized and studied by us, were confirmed through NMR. The racemic *N*-alkyl-phenothiazinyl-ethanols were used to develop analytical methods for the chromatographic separation of the two enantiomers of each alcohol as presented in the Table S1.

Table S1. EKR parameters and chromatographic separation condition of *N*-alkyl-phenothiazine-2-yl-ethan-1-ol (*rac*-**1a-e**) and *N*-alkyl-phenothiazine-3-yl-ethan-1-ol (*rac*-**2b-e**)

Entry	Substrate[a]	Results				
		Yield (%)	*n*-Hexane /IPA (V/V)	Retention time[b]	*c* [c](%)/ Reaction time (h)	ee_S (%)
1	*rac* – **1a**	89	90:10	9.59; 10.19	27.6/12	38.1
2	*rac* – **1b**	47	95:5	7.99; 8.40	49.1/12	96.4
3	*rac* – **1c**	50	75:25	7.14; 9.21	42.6/12	74.2
4	*rac* – **1d**	86	95:5	7.13; 7.46	50/12	>99.9
5	*rac* – **1e**	70	90:10	7.07; 7.50	2.8/12	2.8
6	*rac* – **2b**	24	95:5	9.98; 14.20	40.1/48	67.1
7	*rac* – **2c**	21	70:30	8.42; 15.75	24.9/48	33.1
8	*rac* – **2d**	46	95:5	14.22; 15.04	34.5/48	52.6
9	*rac* – **2e**	75	75:25	6.61; 10.40	19.8/48	24.6

[a] substrate:enzyme mass *ratio* = 10:1; [b]on LUX-i-Cellulose 5 (Cellulose tris(3,5-dichlorophenylcarbamate) chiral column, 250 x 4.6 mm, 3 µm) from Phenomenex, Torrance, CA, USA. [c] ee_P >99% in all cases, E>200 with PVA-CS-CaL-B as catalyst.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.17 – 7.11 (m, 2H), 7.09 (d, *J* = 7.7 Hz, 1H), 6.92 – 6.84 (m, 4H), 4.85 (q, J = 6.4 Hz, 1H), 3.85 (t, J = 7.7 Hz, 2H), 1.80 (t, J = 7.4 Hz, 2H), 1.47 (d, J = 6.4 Hz, 3H), 1.42 (d, J = 6.7 Hz, 2H), 1.32 – 1.27 (m, 4H), 0.89 – 0.84 (m, 3H).

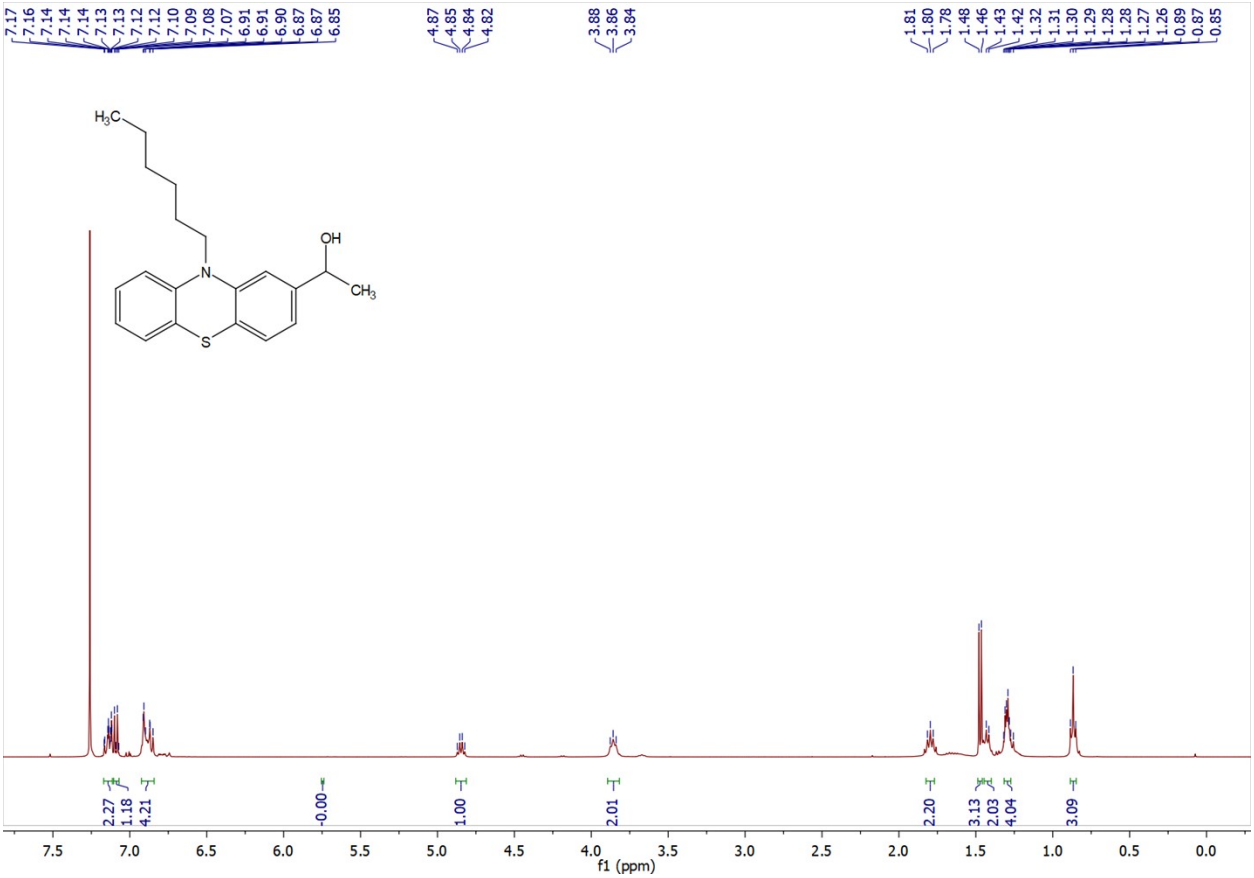


Figure S1: ^{1}H NMR of *rac*-**1e**.

^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.15 – 7.11 (m, 4H), 6.90 (td, *J* = 7.5, 1.2 Hz, 1H), 6.83 (ddd, *J* = 15.8, 7.8, 1.1 Hz, 2H), 4.80 (q, *J* = 6.4 Hz, 1H), 3.84 – 3.81 (m, 2H), 1.81 – 1.75 (m, 2H), 1.46 (d, *J* = 6.4 Hz, 3H), 1.42 (q, *J* = 7.4 Hz, 2H), 1.31 – 1.28 (m, 3H), 0.88 – 0.85 (m, 3H).

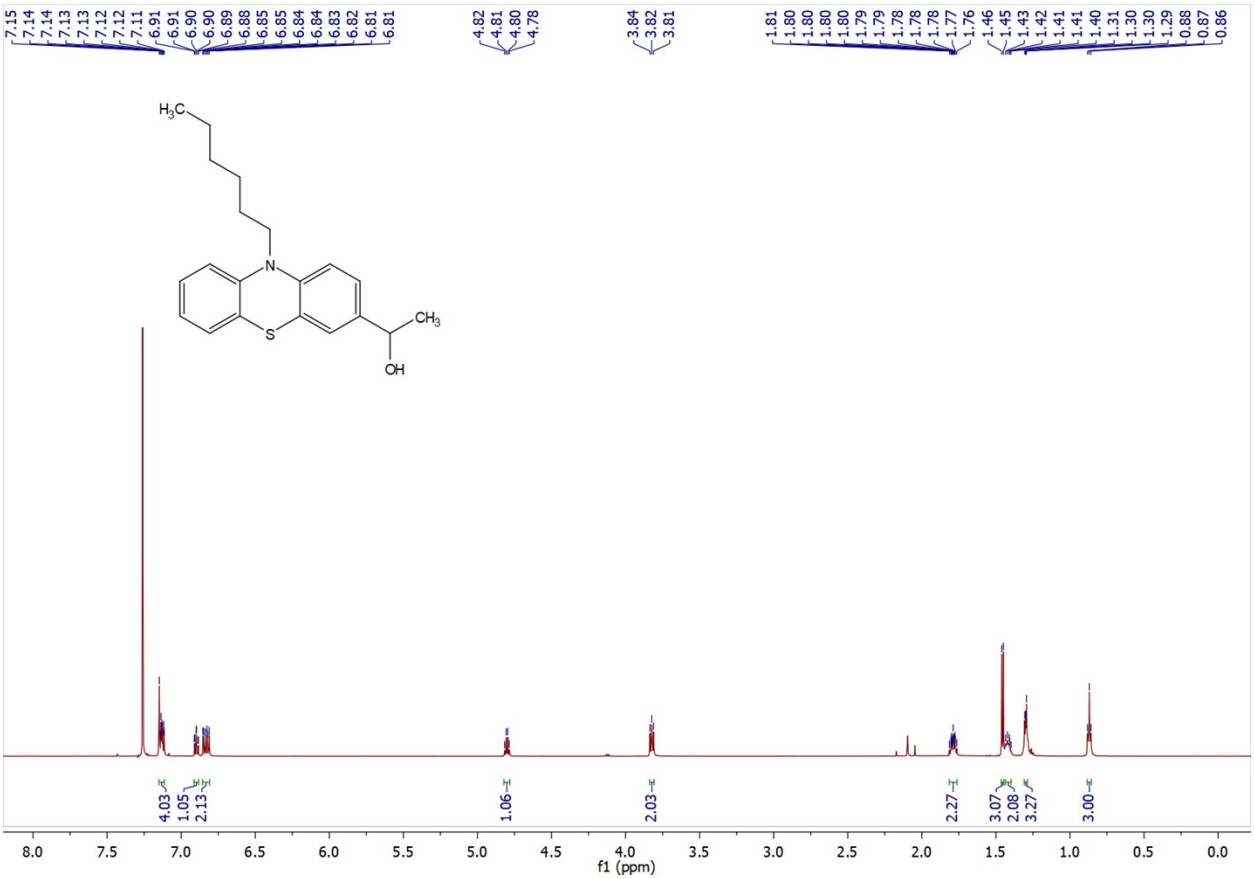
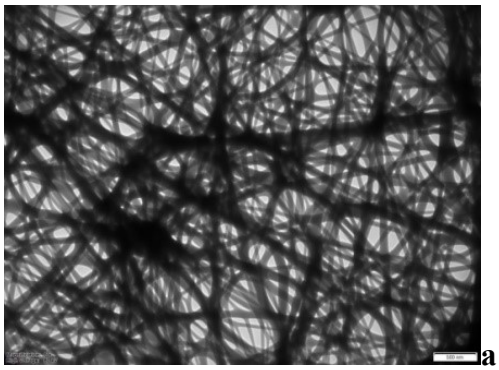
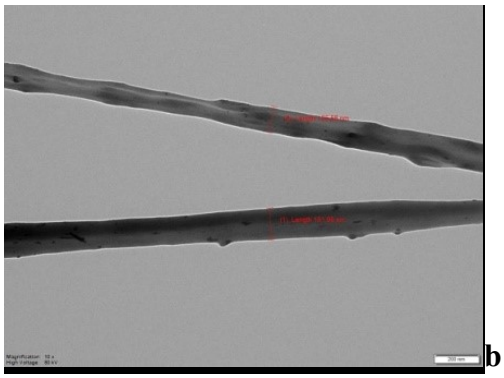


Figure S2: ^{1}H NMR of *rac*-**2e.**



a



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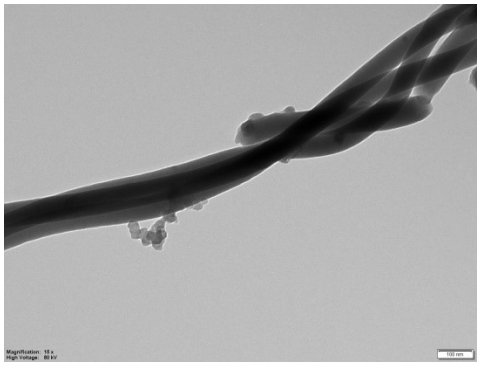

c

Figure S3. TEM images of the new biocatalyst (PVA-CS-CaL-B). **a**. PVA-Chitosan nanofibers homogenity and uniform distribution, with a magnification of 5× (500 nm) and high voltage (80kV); **b**. PVA-Chitosan fibers length determined between 120-152 nm, using a magnification of 10× (200 nm) and high voltage (80kV). **c.** PVA-Chitosan nanofibers containing CaL-B at an amplification allowing to observe enzyme molecules, using a magnification of 15× (100 nm) and high voltage (80kV).

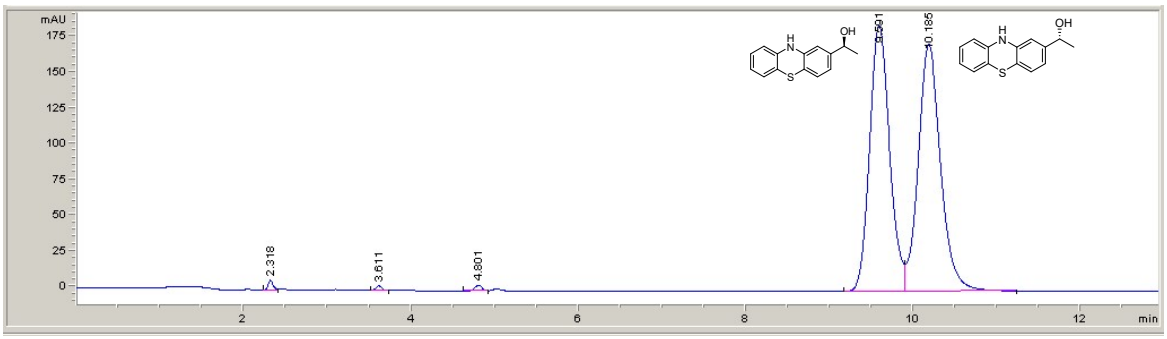

Figure S4. Chromatographic separation of *rac*-**1a** (see Tabel S1 for the chromatographic separation conditions)

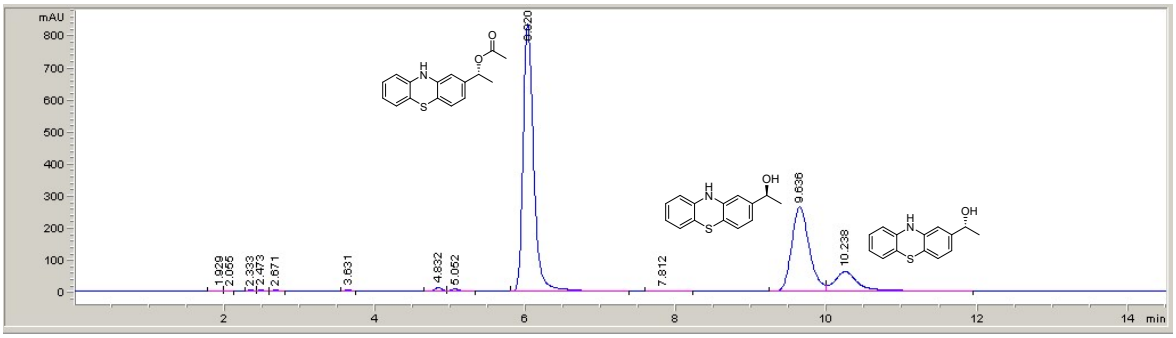

Figure S5. The chromatogram of the reaction mixture of *rac*-**1a** after 12 h (see Tabel S1 for the chromatographic separation conditions).

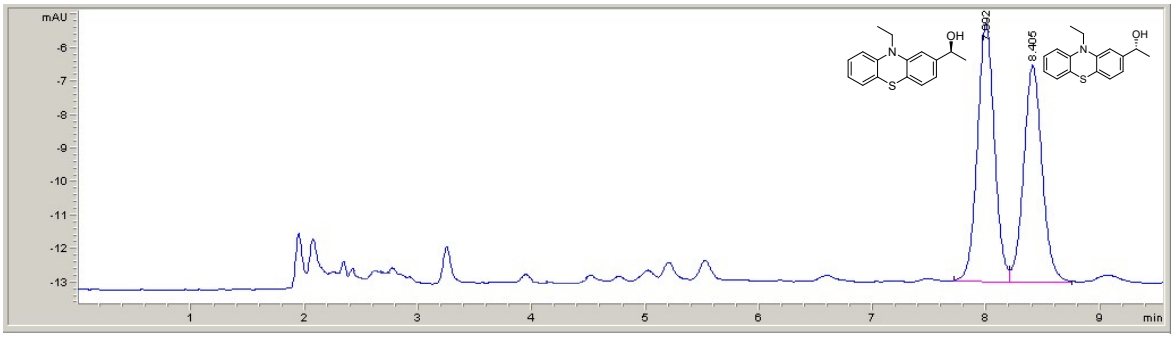

Figure S6. Chromatographic separation of *rac*-**1b** (see Tabel S1 for the chromatographic separation conditions).

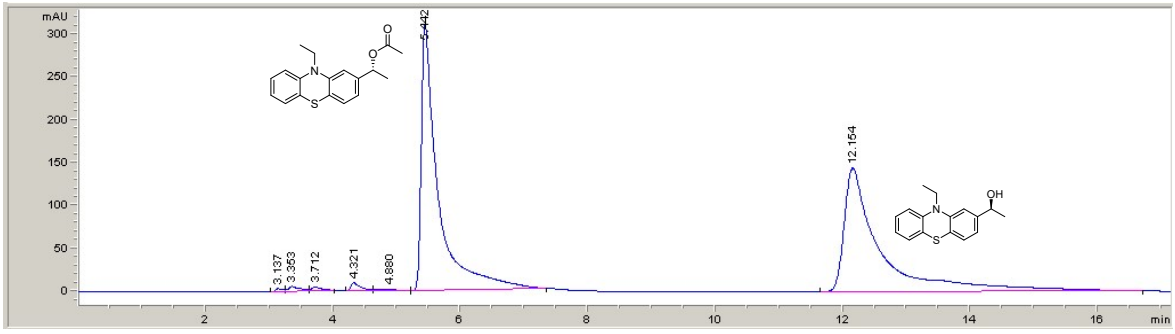

Figure S7. The chromatogram of the reaction mixture of *rac*-**1b** after 12 h (see Tabel S1 for the chromatographic separation conditions).

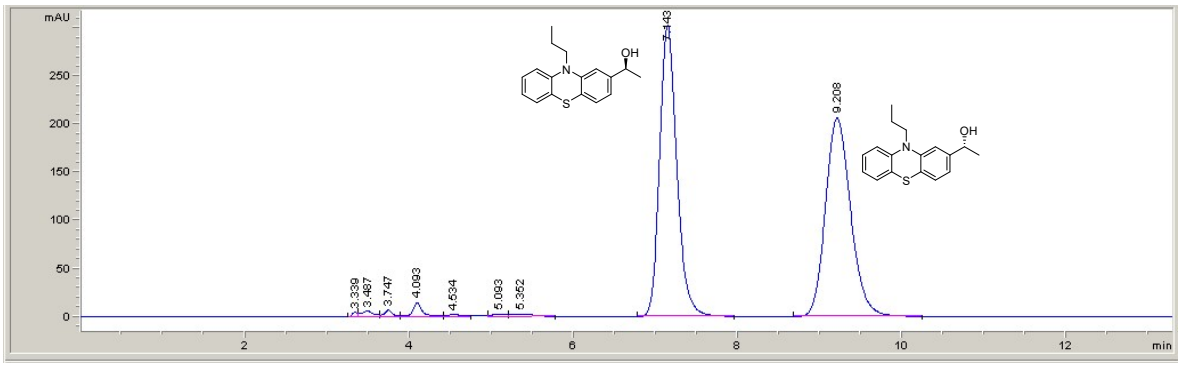

Figure S8. Chromatographic separation of *rac*-**1c** (see Tabel S1 for the chromatographic separation conditions).

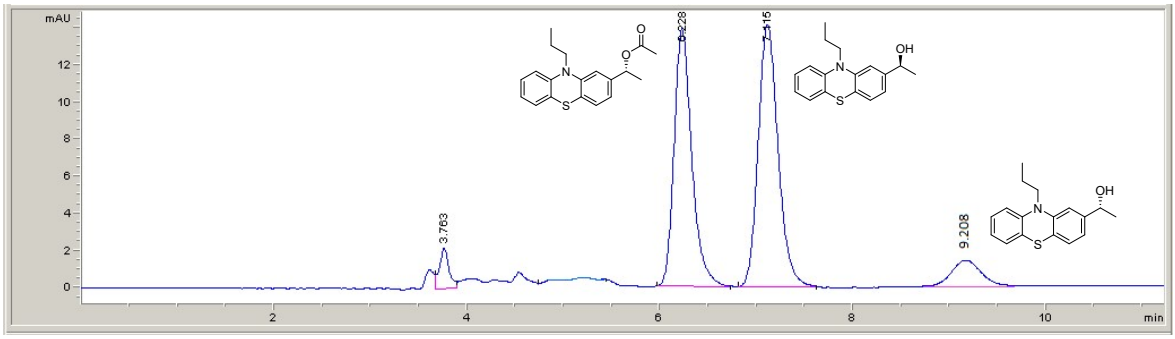

Figure S9. The chromatogram of the reaction mixture of *rac*-**1c** after 12 h (see Tabel S1 for the chromatographic separation conditions).

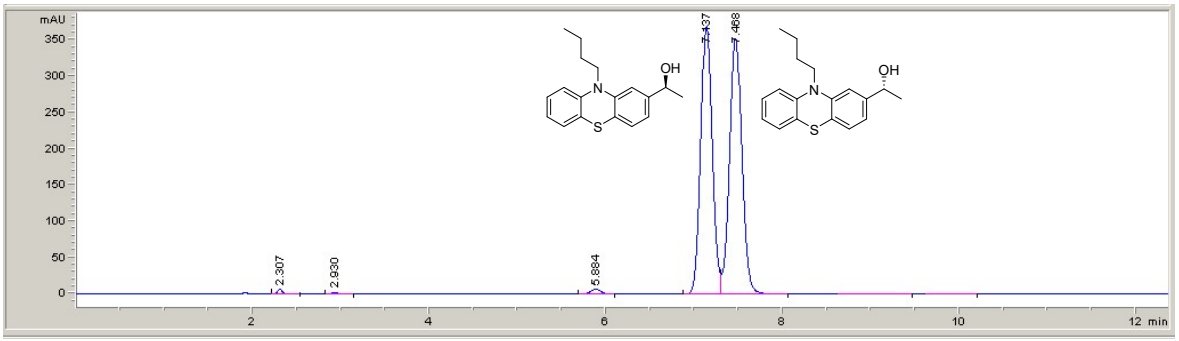

Figure S10. Chromatographic separation of *rac*-**1d** (see Tabel S1 for the chromatographic separation conditions).

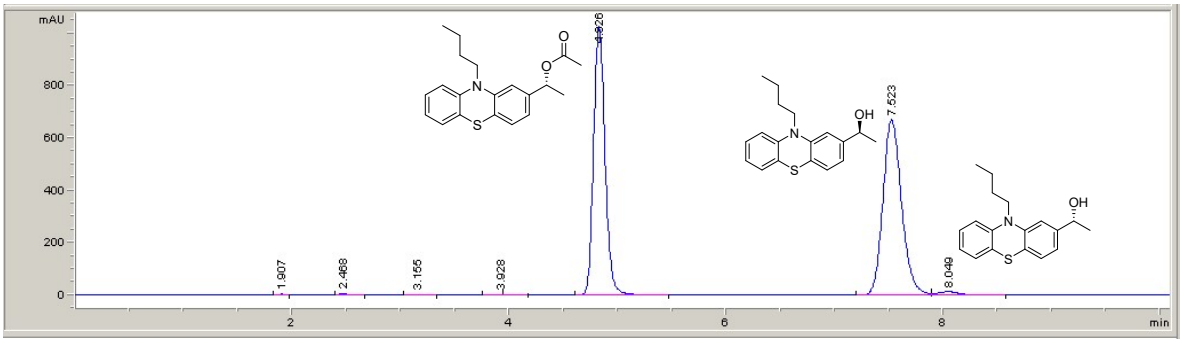

Figure S11. The chromatogram of the reaction mixture of *rac*-**1d** after 12 h (see Tabel S1 for the chromatographic separation conditions).

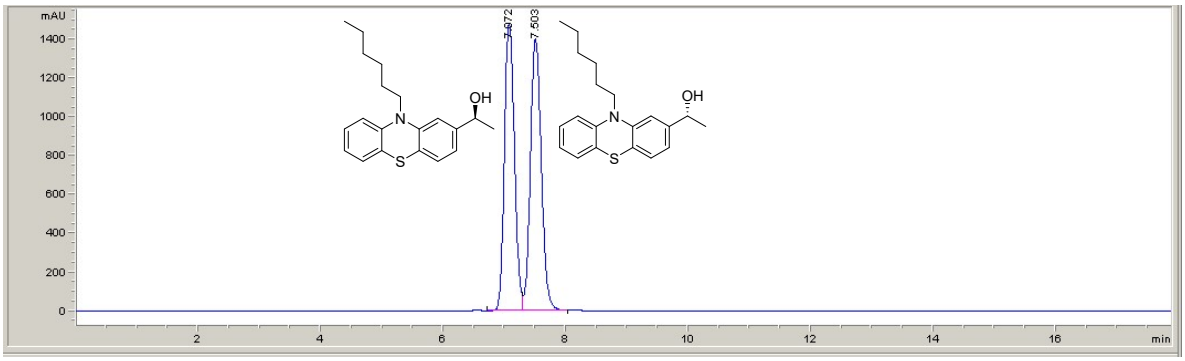

Figure S12. Chromatographic separation of *rac*-**1e** (see Tabel S1 for the chromatographic separation conditions).

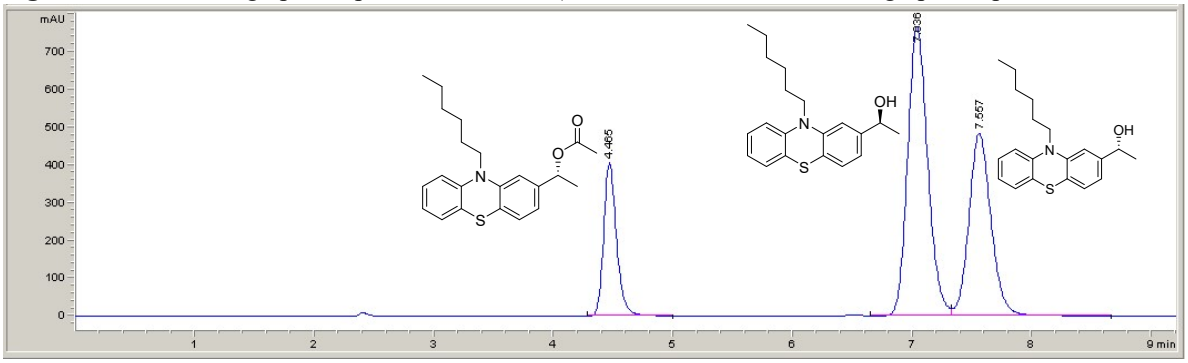

Figure S13. The chromatogram of the reaction mixture of *rac*-**1e** after 12 h (see Tabel S1 for the chromatographic separation conditions).

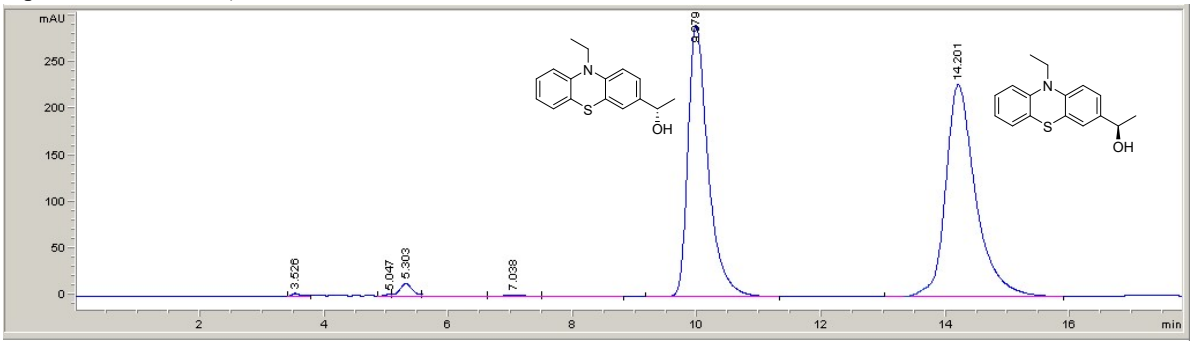

Figure S14. Chromatographic separation of *rac*-**2b** (see Tabel S1 for the chromatographic separation conditions).

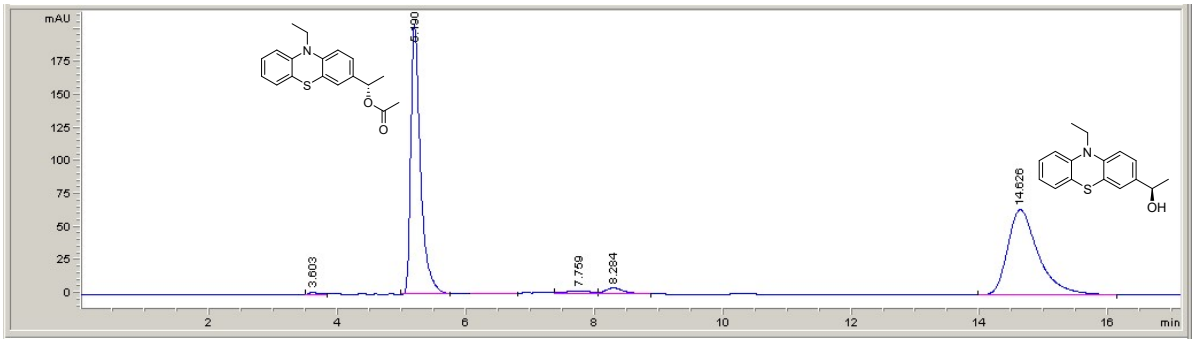

Figure S15. The chromatogram of the reaction mixture of *rac*-**2b** after 48 h (see Tabel S1 for the chromatographic separation conditions).

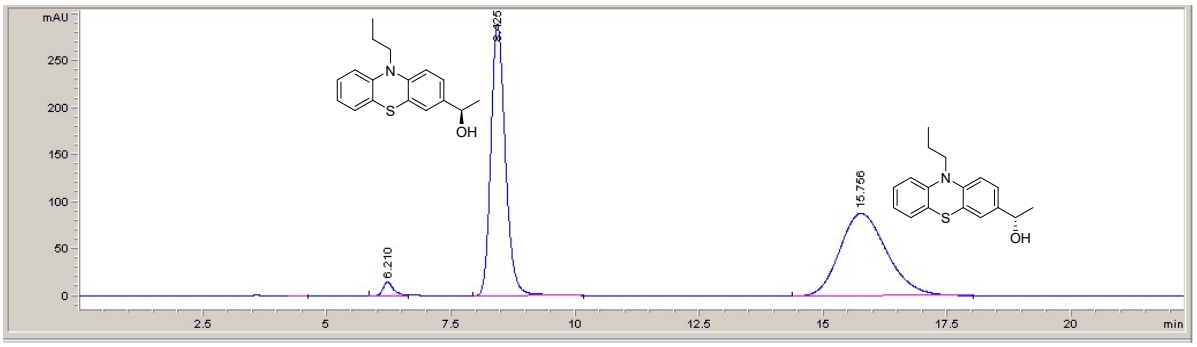

Figure S16. Chromatographic separation of *rac*-**2c** (see Tabel S1 for the chromatographic separation conditions).

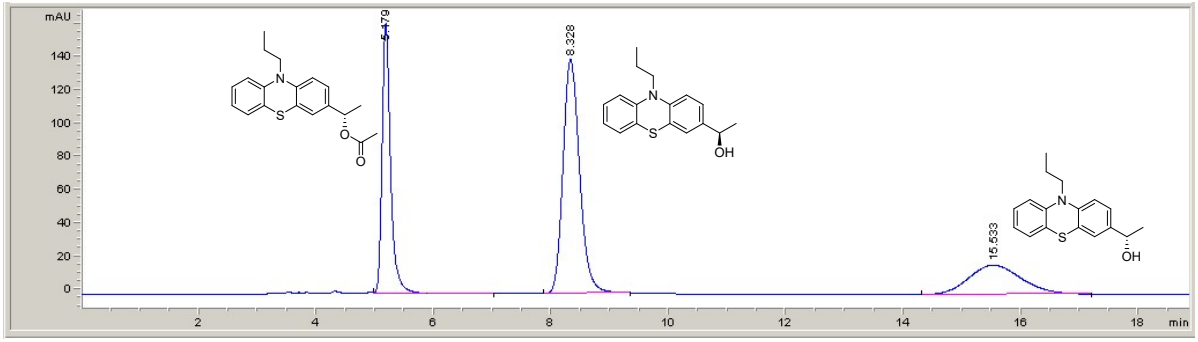

Figure S17. The chromatogram of the reaction mixture of *rac*-**2c** after 48 h (see Tabel S1 for the chromatographic separation conditions).

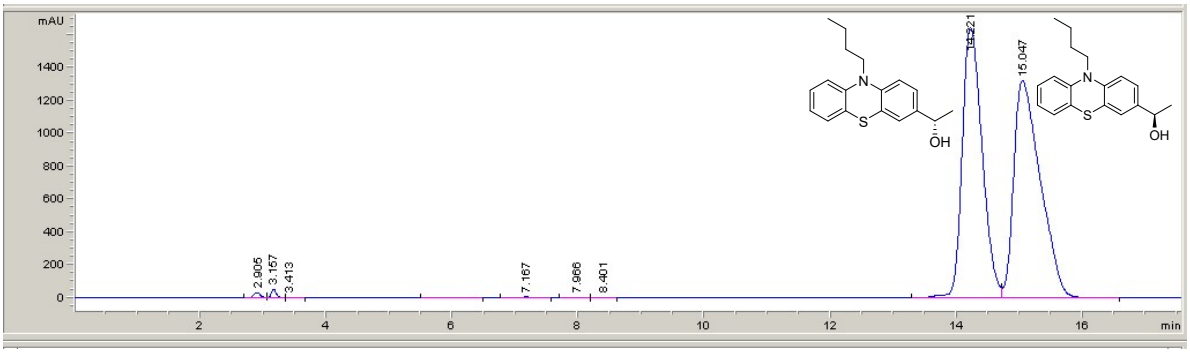

Figure S18. Chromatographic separation of *rac*-**2d** (see Tabel S1 for the chromatographic separation conditions).

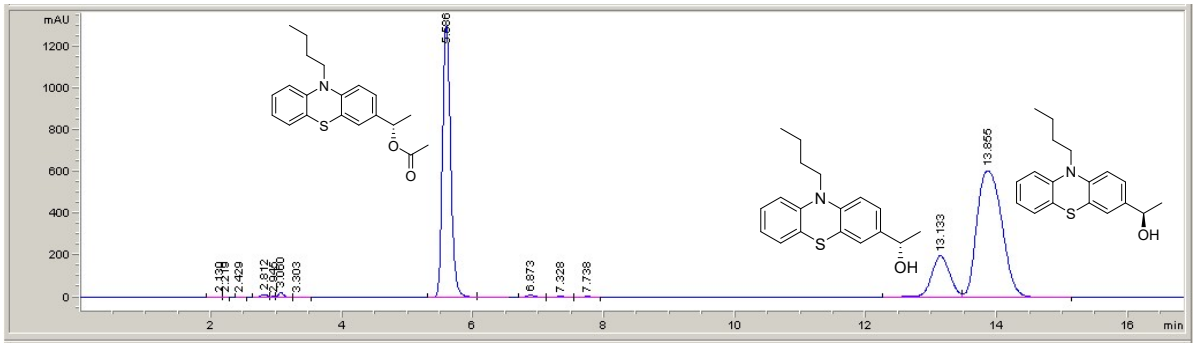

Figure S19. The chromatogram of the reaction mixture of *rac*-**2d** after 48 h (see Tabel S1 for the chromatographic separation conditions).

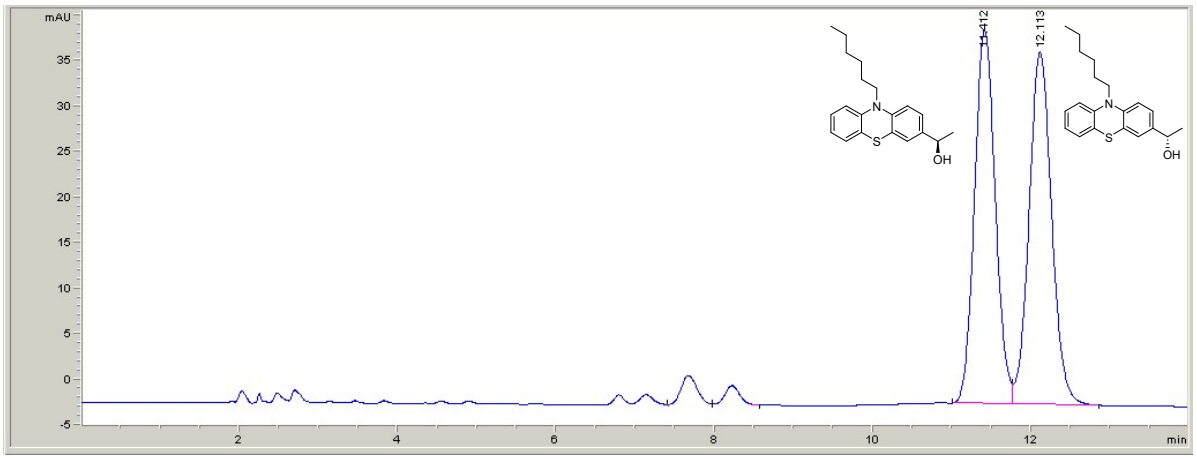

Figure S20. Chromatographic separation of *rac*-**2e** (see Tabel S1 for the chromatographic separation conditions).

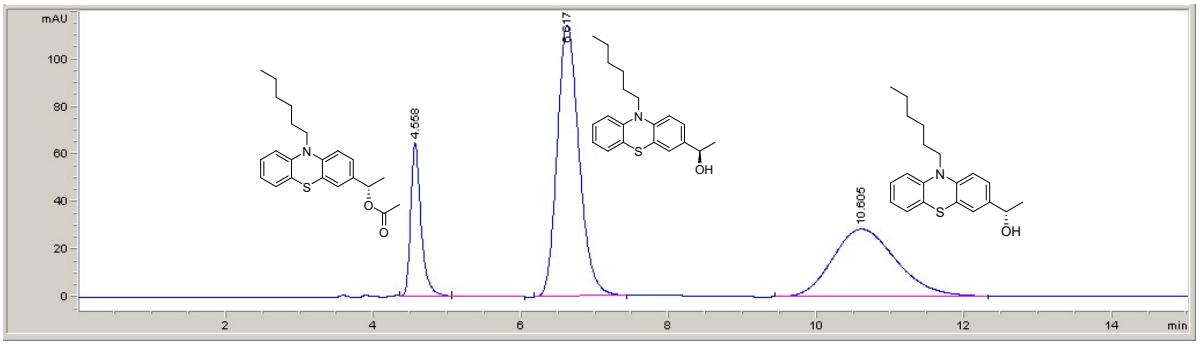

Figure S21. The chromatogram of the reaction mixture of *rac*-**2e** after 48 h (see Tabel S1 for the chromatographic separation conditions).

References

1. C. Paizs, M.-I. Tosa, V. Bódai, G. Szakács, I. Kmecz, B. Simándi, C. Majdik, L. Nová, F.-D. Irimie and L. Poppe, *Tetrahedron: Asymmetry***, 2003**, *14,* 1943.
2. M.-I. Tosa, S. Pilbák, P. Moldovan, C. Paizs, G. Szatzker, G. Szakács, L. Novák, F.-D. Irimie and L. Poppe, *Tetrahedron: Asymmetry* **2008**, *19*, 1844.
3. J. Brem, M.-I. Tosa, C. Paizs, A. Munceanu, D. Matcović-Čalogović and F.-D. Irimie, *Tetrahedron: Asymmetry* **2010**, *21*, 1993.