STUDIA UBB. CHEMIA, LV, 4, 2010

SYNTHESIS AND LIPASE CATALYSED KINETIC RESOLUTION OF RACEMIC AMINES

PÉTER FALUS[a], ZOLTÁN BOROS[a], GÁBOR HORNYÁNSZKY[a], JÓZSEF NAGY[a], LÁSZLÓ ÜRGE[b], FERENC DARVAS[c], LÁSZLÓ POPPE[a]*

ABSTRACT. Feasibility of production of amines from ketones employing metal and/or metal-catalysts in one-pot and one-step reductive amination (modified Leuckart- and Leuckart-Wallach- reaction) and lipase catalysed kinetic resolution of racemic amines in batch and continuous-flow reactor were investigated. In kinetic resolutions the effect of the solvent, the acetylating agent and the lipase itself was examined.

Keywords: *reductive amination, metal-catalysis, lipase, continuous-flow reactor, kinetic resolution*

INTRODUCTION

Enantiomerically pure chiral amines are valuable building blocks of quite a number of drugs [1], pesticides [2] and colour pigments [3]. Considerable amount of drugs are amines or amine derivatives.

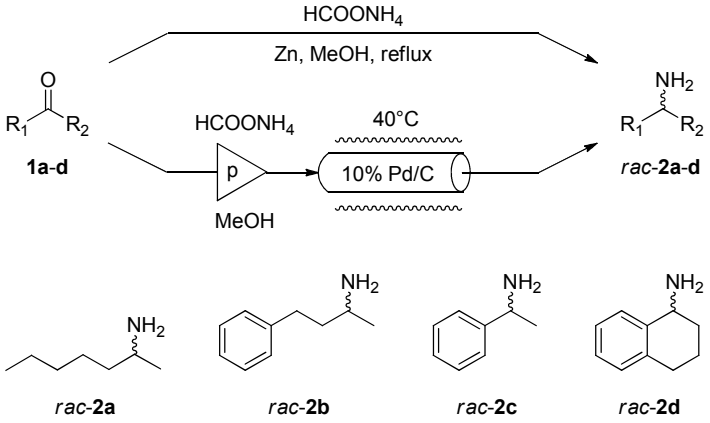
Biotechnology and biocatalysis are increasingly employed to produce optically active intermediates of drugs [4]. Hydrolases can be efficiently used for synthetic biotransformations due to its favourable characteristics [5, 6, 7, 8]. Hydrolases can catalyze several related reactions such as hydrolysis, condensations, alcoholysis and aminolysis. Lipases are proved to be highly versatile biocatalyst in stereoselective biotransformations such as kinetic resolutions [9], deracemisations and dynamic kinetic resolutions [10]. Enantioselective enzymatic reactions are typically carried out in batch mode [5, 9, 11, 12], however a few studies state these are feasible in continuous-flow system [13, 14, 15, 16].

RESULTS AND DISCUSSION

In our previous work, one-step reductive amination of various ketones was examined [17]. It was found that in those cases when the carbonyl-group is at α-position from an aromatic or heteroaromatic ring Zn dust promoted reactions gave the corresponding amines without notable side reactions, whereas in the case of aliphatic and cycloaliphatic ketones 10% Pd/C-catalysis was suitable.

[a] *Budapest University of Technology and Economics, Department of Organic Chemistry and Technology, and Research Group for Alkaloid Chemistry of HAS, Műegyetem rkp 3, H-1111, Budapest, Hungary, * poppe@mail.bme.hu*
[b] *ThalesNano Inc., Graphisoft Park, Záhony u. 7., H-1031 Budapest, Hungary*
[c] *Department of Cellular Biology and Pharmacology, Herbert Weinheim College of Medicine, Florida International University, 11200 S.W. 8th Street, Miami, FL 33199, USA*

The amines **2a-d** for the enzymatic studies were prepared by the reductive amination of the corresponding ketone **1a-d** by our novel method [17] (Scheme 1). Aliphatic ketones **1a,b** were treated with ammonium formate in methanol at 40ºC until the disappearance of the starting ketone. The reaction was catalysed by 10% Pd/C and was performed in continuous-flow reactor. Conversely, Zn dust proved to be an effective catalyst for the transformation of carbonyl groups at benzylic sidechain position of aromatic system **1c,d** at the reflux temperature of methanol in batch reaction (Scheme 1). The following yields were achieved: 53% for *rac*-**2a**, 41% for *rac*-**2b**, 71% for *rac*-**2c** and 71% for *rac*-**2d**.



Scheme 1

Herein, we intended to study the effects of solvent and nature of the biocatalyst on the lipase catalysed kinetic resolution of four racemic amines **2a-d**. For selecting the proper catalyst for the continuous-flow mode kinetic resolution various lipases (immobilized and non-immobilized) were screened in batch mode. The two examined CalB (*Candida antarctica* Lipase B) enzymes – immobilized on polymeric carriers by two different methods – resulted in formation of (*R*)-*N*-acetamides in high enantiomeric excesses (*ee*) with moderate to good conversions (*c*). Our in house made BUTE 3 (F-4) [18] biocatalyst also gave acceptable enantiomeric excess and conversion in certain cases.

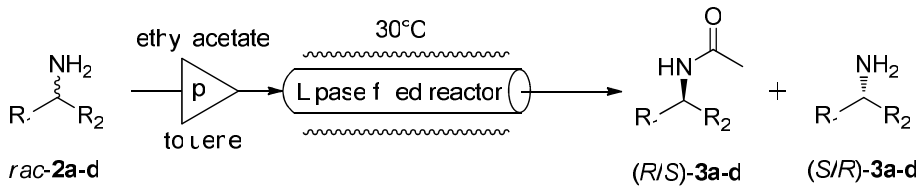
Efficiency of the biocatalytic reactions are highly influenced by the milieu. Thus, different solvents (toluene, trifluorotoluene, *tert*-butyl methyl ether, diisopropyl ether, ethyl acetate, hexane, tetrahydrofuran, hexane-tetrahydrofuran 2:1) and acetylating agents (ethyl acetate, isopropenyl acetate, ethylene glycol diacetate) were examined next. The best results (*ee* and *c*) were reached with the use of ethyl acetate as acetylating agent in toluene (representative results in toluene are in Table 1). It was also noticed that the use of ether-like solvents gave high enantiomeric excesses, however with lower conversions (data not shown). Our study also revealed, that the nature of immobilization of lipase B from *Candida antarctica* (Novozym 435 vs. CalB T2-150) had remarkable effects on the activity and selectivity of the enzyme (Table 1).

Table 1. Kinetic resolution of racemic amines **2a-d** with ethyl acetate in toluene

Substrate	Enzyme	Time [h]	*c* [%][a]	$ee_{(R)\text{-}3a}$ [%][a]	$E_{c,ee(P)}$ [%][b]
2a	Novozym 435	8	25.4	98.0	>100
2a	CalB T2-150	8	11.5	97.9	>100
2a	Novozym 435	24	37.0	96.0	86
2a	CalB T2-150	24	19.7	97.1	85
2b	Novozym 435	8	39.8	97.4	>100
2b	CalB T2-150	8	28.9	98.7	>200
2b	BUTE 3 (F-4)	8	18.7	97.7	>100
2b	Novozym 435	24	52.7	96.4	-[c]
2b	CalB T2-150	24	43.5	96.8	>100
2b	BUTE 3 (F-4)	24	28.0	98.4	>100
2c	Novozym 435	8	12.0	95.5	50
2c	CalB T2-150	8	3.2	85.3	13
2c	Novozym 435	24	25.5	94.2	46
2c	CalB T2-150	24	8.1	88.3	17
2d	CalB T2-150	8	3.3	94.3	35
2d	Novozym 435	8	16.4	96.4	66
2d	CalB T2-150	24	9.1	92.2	27
2d	Novozym 435	24	32.1	95.0	61

[a] Determined by chiral phase GC;
[b] Calculated by using *c* and $ee_{(R)\text{-}3a}$ [19]. Due to sensitivity to experimental errors, *E* values calculated in the 100–250 range are reported as >100, values above 250 are given as >200;
[c] Enantiomeric selectivity can not be calculated properly above 50% conversion [19].

Finally, kinetic resolutions of racemic amines **2a-d** were performed at preparative scale in X-Cube reactor operating in continuous-flow mode. The solution of the corresponding amine **2a-d** in toluene-ethyl acetate 9:1 was pumped through a Novozym 435 filled CatCart column thermostated to 30ºC at a flow rate of 0.5 mL min^{-1} (Scheme 2).



Scheme 2

After the stationary state reached (~8 times dead volume of the column, 40 min), feeding the reactor with amine **2a-d** was continued for 6 h. The *N*-acetamide **3a-d** was isolated from the collected homogenous solutions (Table 2). In all cases, higher enantiomeric excesses and conversions were obtained in continuous-flow reactor than in the corresponding batch reaction. The main advantage of the continuous-flow system with lipase filled-columns is the recyclability and the efficient and reproducible use of the catalyst. This was demonstrated by repeating the 6 h long reactions of amines **2a-d** in a second series of 6 h long reactions using the same Novozym 435-filled column with the same results.

Table 2. Kinetic resolution of racemic amines **2a-d** by Novozyme 435 in continuous-flow reactor

Substrate	c [%][a]	$ee_{(R)\text{-}3}$ [%][b]	$E_{c,ee(P)}$ [%][c]
2a	43.3	97.9	>200
2b	47.2	98.8	>200
2c	48.1	98.7	>200
2d	45.7	99.3	»200

[a] Determined after removal of the solvent from the resulting mixture;
[b] Determined by chiral phase GC;
[c] Calculated by using c and $ee_{(R)\text{-}3}$ [19]. Due to sensitivity to experimental errors, E values calculated in the 100–250 range are reported as >100, values in the 250–500 range are reported as >200 and values calculated above 500 are given as »200

CONCLUSIONS

In this study, solvent and catalyst effects on the kinetic resolutions of racemic amines **2a-d** (synthesized from the corresponding ketones **1a-d**) in batch and in continuous flow mode were investigated. It was found that immobilized forms of lipase B from (Novozym 435 and CalB T-2 150) are the most suitable biocatalysts in acylations with ethyl acetate in toluene with the examined substrates **2a-d**. Comparison of the batch and continuous mode reactions indicated that the continuous-flow process was superior to the corresponding batch reaction in cases of all the four (*R*)-acetamides (*R*)-**3a-d**.

EXPERIMENTAL SECTION

Analytical methods

The NMR spectra were recorded in DMSO on a Bruker DRX-500 spectrometer and are reported in ppm on the δ scale. Infrared spectra were recorded on a Bruker ALPHA FT-IR spectrometer. TLC was carried out on Kieselgel 60F254 (Merck) sheets. Spots were visualized under UV light (Vilber Lourmat VL-6.LC, 254 nm and 365 nm) or by treatment with 5% ethanolic phosphomolybdic acid solution and heating of the dried plates. GC analyses were carried out on Younglin ACME 6100 or Agilent 4890D instruments equipped with FID detector and Hydrodex-β-TBDAc column (50 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-*O*-acetyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrin; Macherey&Nagel) or Hydrodex-β-6TBDM column (25 m × 0.25 mm × 0.25 μm film with heptakis-(2,3-di-*O*-methyl-6-*O*-*t*-butyldimethylsilyl)-β-cyclodextrine; Macherey&Nagel) using H_2 carrier gas (injector: 250°C, detector: 250°C, head pressure: 10 psi, 50:1 split ratio). The continuous flow reactions were performed by X-Cube™ laboratory flow reactor (X-Cube™ - trademark of ThalesNano, Inc.; Ser. No.: 002/2006) equipped with 10% Pd/C [THS 01111] or Novozym 435 [THS 01724] filled CatCart™ columns (CatCart™ – registered trademark of ThalesNano Inc.: cat. no.: THS X1175; stainless steel (INOX 316L); inner diameter: 4 mm; total length: 70 mm; packed length: 65 mm; inner volume: 0.816 mL).

Chemicals and enzymes

Heptan-2-one **1a**; 4-phenylbutan-2-one **1b**; acetophenone **1c**; 3,4-dihydronaphthalen-1(2*H*)-one **1d**; ammonium formate and all further chemicals and solvents were of analytical grade or higher were products of Sigma, Aldrich, Fluka, Alfa Aesar or Merck. 10% Pd/C [THS 01111] and Novozym 435 [THS 01724] filled CatCart^{TM} columns were products of ThalesNano, Inc. Lipase AK, Lipase PS, Lipase AYS were obtained from Amano Europe. Novozym 435 was purchased from Novozymes, Denmark. *Mucor miehei* lipase, Lipase PPL, Lipase CcL, *Candida rugosa* were products of Sigma. Lipase AY, Lipase M were obtained from Amano Pharmaceutical. Lipozyme TL IM was purchased from Novo Nordisk A/S. CalB T2-150, CalA T2-150, CRL T2-150, IMMAULI T2-150 were products of Chiral Vision. Lipobond-Lipase PS was a kind gift of Iris Biotech GmbH. The sol-gel immobilzed lipases (sol-gel lipase AK: 338b and 251b; sol-gel lipase PS) [20], lipases from thermophilic fungi (BUTE 3 (T-2), BUTE (F-4)) [18] and lipases from solid-state fermentations (SSF9, SSF23) [21] were prepared in our laboratory.

General procedure of the continuous-flow reductive amination of ketones (***1a,b***) *[17]*

The solution of the corresponding ketone (5 mg mL^{-1} of **1a,b**: 0.044 mmol mL^{-1} of **1a**, 0.034 mmol mL^{-1} of **1b**) and 6 equiv. ammonium formate (16.65 mg mL^{-1} for **1a**, 12.86 mg mL^{-1} for **1b**) in methanol was pumped through the 10% Pd/C filled column thermostated to 40°C at a flow rate of 0.2 mL min^{-1} without choking (no measurable back-pressure). After the stationary state reached (approximately 8x the whole volume of the system) the mixture was pumped through the column for 6 hours. After a run the columns were routinely washed with methanol (0.5 mL min^{-1}, 20 min). The collected reaction mixture was concentrated under reduced pressure. The residue was treated with conc. HCl solution (3 mL) and water (20 mL) and extracted with diethyl ether (2x15 mL). The aqueous phase was treated with ammonia solution until pH=10 and extracted with dichloromethane (4x20 mL). The organic phase was treated with brine, dried over sodium sulfate and the solvent was distilled off from the resulting solution by rotary evaporation to give the amines **2a-d**.

racemic Heptan-2-amine *rac*-**2a**: pale yellow liquid; ^{1}H NMR (300 MHz, DMSO-d_6): 0.86 (3H, t, J=7.0 Hz, CH_3); 0.93 (3H, d, J=6.2 Hz, CH_3); 1.11-1.36 (8H, m, 4×CH_2); 2.93 (brs, NH_2+H_2O); 2.71 (1H, m, J=6.3 Hz, CHN); ^{13}C NMR (75 MHz, DMSO-d_6): 14.47 (CH_3); 22.70 (CH_2); 24.49 (CH_3); 26.08 (CH_2); 32.10 (CH_2); 40.29 (CH_2); 46.87 (CH); IR (cm^{-1}): 3325, 3284, 2956, 2927, 2858, 1564, 1456, 1362, 1294, 1166, 1097, 855, 814, 725

racemic 4-Phenylbutan-amine *rac*-**2b**: colourless liquid; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.00 (3H, d, J=6.3 Hz, CH_3); 1.43-1.58 (4H, brs+m, NH_2+CH_2); 2.50-2.70 (2H, m, CH_2); 2.74 (1H, q, J=6.3 Hz, CHN); 7.12-7.29 (5H, m, 5×CH); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 24.09 (CH_3); 32.10 (CH_2); 41.74 (CH_2); 45.92 (CH); 125.42 (CH); 128.14 (2×CH); 128.16 (2×CH); 142.51 (C); IR (cm^{-1}): 3412, 3396, 2956, 2925, 2858, 1561, 1458, 1363, 1302, 1163, 886, 854, 815, 723, 460

*General procedure for the reduction of ketones **1c,d** to amines **2c,d** in one-step batch synthesis [17]*

A mixture of the ketone **1c,d** (10 mmol: 1.46 g of **1c**, 1.20 g of **1d**), ammonium formate (60 mmol, 3.78 g) and Zn powder (30 mmol, 1.96 g) in methanol (30 mL) was stirred under reflux until disappearance of the ketone (monitored by TLC). The reaction mixture was strained through Celite®, and the solvent was removed under vacuum. The residue was treated with conc. HCl solution (4 mL) and water (30 mL) and extracted with diethyl ether (2x20 mL). The aqueous phase was treated with ammonia solution until pH=10 and extracted with dichloromethane (4x25 mL). The organic phase was treated with brine, dried over sodium sulfate and the solvent was distilled off from the resulting solution by rotary evaporation.

racemic 1-Phenylethanamine *rac*-**2c**: colourless liquid; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.24 (3H, d, J=6.6 Hz, CH_3); 3.02 (brs, NH_2+H_2O); 3.97 (1H, q, J=6.6 Hz, CHN); 7.18 (1H, m, CH); 7.29 (2H, m, 2×CH); 7.36 (2H, m, 2×CH); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 26.69 (CH_3); 51.16 (CH); 126.25 (2×CH); 126.64 (CH); 128.57 (CH); 149.26 (C); IR (cm^{-1}): 3362, 3301, 3061, 3026, 2965, 2924, 2868, 1604, 1579, 1492, 1475, 1450, 1364, 1024, 859, 762, 698, 591, 537

racemic 1,2,3,4-Tetrahydronaphthalen-1-amine *rac*-**2d**: yellow liquid; ^{1}H NMR (300 MHz, DMSO-d_6): 1.47-1.72 (2H, m, CH_2); 1.78-1.96 (2H, m, CH_2); 2.57-2.78 (2H, m, CH_2); 3.02 (brs, NH_2+H_2O); 3.79 (1H, t, J=6.2 Hz, CHN); 6.98-7.16 (3H, m, 3×CH); 7.40-7.48 (1H, m, CH); ^{13}C NMR (75 MHz, DMSO-d_6): 19.97 (CH_2); 29.67 (CH_2); 33.76 (CH_2); 49.41 (CH); 125.96 (CH); 126.42 (CH); 128.61 (CH); 128.89 (CH); 136.73 (C); 142.39 (C); IR (cm^{-1}): 3412, 3381, 3057, 3015, 2924, 2858, 1578, 1488, 1447, 1371, 1337, 1155, 884, 854, 761, 733, 584, 433

*Enantiomer selective acetylation of racemic amines **2a-d** in shake vials*

To a solution of the racemic amine **2a-d** (20 mg) in toluene-ethyl acetate 9:1 mixture (1 mL), the enzyme (20 mg) was added in a sealed amber glass vial and the resulting mixture was shaken (1000 rpm) at 30°C for 24 h. The reactions were analyzed by GC and TLC after 1, 2, 4, 8 and 24 hours.

*Enantiomer selective acetylation of racemic amines **3a-d** in continuous mode*

The solution of racemic amine **2a-d** (10 mg mL^{-1}) in toluene-ethyl acetate 9:1 mixture was pumped through three serially connected Novozym 435-filled CatCart columns operated at 30ºC and the product was collected at a flow rate of 0.5 mL min^{-1} without choking. After 40 min (which was necessary to reach the stationary state of the bioreactor) the reaction mixture was collected for 6 h. The solvent was removed from the reaction mixture by vacuum rotary evaporation. The residue was dissolved in dichloromethane (100 mL), treated with 5% HCl solution (10 mL) and the aqueous phase was extracted with dichloromethane (4x3 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under vacuum. The aqueous phase was alkalized with ammonia solution to pH=10 and extracted with dichloromethane (3x10 mL). The organic phase was dried over Na_2SO_4 and concentrated under vacuum.

(*R*)-*N*-(Heptan-2-yl)acetamide (*R*)-**3a**: yellow oil; ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.85 (3H, t, J=7.0 Hz, CH_3); 0.99 (3H, d, J=6.6 Hz, CH_3); 1.15-1.40 (8H, m, 4×CH_2); 1.76 (3H, s, CH_3); 3.70 (1H, m, CHN); 7.62 (1H, br d, J=8.1 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm): 14.38 (CH_3); 21.22 (CH_3); 22.52 (CH_2); 23.14 (CH_3); 25.86 (CH_2); 31.72 (CH_2); 36.57 (CH_2); 44.54 (CH); 168.76 (CO); IR (cm^{-1}): 3275, 3079, 2959, 2928, 2858, 1638, 1550, 1453, 1371, 1293, 1157, 974, 726, 608

(*R*)-*N*-(4-Phenylbutan-2-yl)acetamide (*R*)-**3b**: pale yellow liquid; ^{1}H NMR (300 MHz, DMSO-d_6): 1.04 (3H, d, J=6.6 Hz, CH_3); 1.57-1.71 (2H, m, CH_2); 1.81 (3H, s, CH_3); 2.48-2.61 (2H, m, CH_2); 3.75 (1H, m, CHN); 7.11-7.21 (3H, m, 3×CH); 7.22-7.31 (2H, m, 2×CH); 7.74 (1H, br d, J=8.1 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 21.24 (CH_3); 23.24 (CH_3); 32.42 (CH_2); 38.44 (CH_2); 44.42 (CH); 126.09 (CH); 128.83 (4×CH); 142.36 (C); 168.93 (CO); IR (cm^{-1}): 3271, 3084, 3065, 2967, 2929, 1637, 1546, 1495, 1453, 1371, 1292, 1144, 967, 746, 698, 609

(*R*)-*N*-(1-Phenylethyl)acetamide (*R*)-**3c**: tawny crystals; ^{1}H NMR (300 MHz, DMSO-d_6): 1.33 (3H, d, J=7.2 Hz, CH_3); 1.84 (3H, s, CH_3); 4.90 (1H, m, CHN); 7.14-7.26 (1H, m, CH); 7.27-7.36 (4H, m, 4×CH); 8.30 (1H, br d, J=7.9 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 22.47 (CH_3); 22.64 (CH_3); 47.78 (CH); 125.93 (2×CH); 126.64 (CH); 128.28 (2×CH); 144.87 (C); 168.30 (CO); IR (cm^{-1}): 3266, 3071, 3023, 2980, 2929, 1643, 1555, 1451, 1375, 1286, 1278, 1216, 1136, 1070, 1027, 763, 702, 620, 533, 501

(*R*)-*N*-(1,2,3,4-Tetrahydronaphthalen-1-yl)acetamide (*R*)-**3d**: brown crystals; ^{1}H NMR (300 MHz, DMSO-d_6): 1.58-1.78 (2H, m, CH_2); 1.79-1.95 (5H, m, CH_3+CH_2); 2.63-2.82 (2H, m, CH_2); 4.90-5.03 (1H, m, CHN); 7.04-7.20 (4H, m, 4×CH); 8.22 (1H, br d, J=8.5 Hz, NH); ^{13}C NMR (75 MHz, DMSO-d_6): 20.47 (CH_2); 23.20 (CH_3); 29.25 (CH_2); 30.48 (CH_2); 46.86 (CH); 126.28 (CH); 127.22 (CH); 128.67 (CH); 129.28 (CH); 137.45 (C); 138.07 (C); 169.05 (CO); IR (cm^{-1}; KBr): 3240, 3062, 2928, 2854, 1633, 1544, 1445, 1371, 1283, 1095, 1038, 965, 764, 739, 610, 536, 446

ACKNOWLEDGEMENTS

This research work was supported by the Hungarian National Office for Research and Technology (NKFP-07-A2 FLOWREAC). This work is also related to the scientific program of "*Development of quality-oriented and harmonized R+D+I strategy and functional model at BME*" project (TÁMOP-4.2.1/B-09/1/KMR-2010-0002), supported by the New Hungary Development Plan. The authors thank Dr. György Szakács (Budapest University of Technology and Economics, Hungary) and Dr. Balázs Erdélyi (Fermentia Ltd, Budapest, Hungary) for the BUTE 3 lipase preparations.

REFERENCES

1. T. Henkel, R.M. Brunne, H. Müller, F. Reichel, *Angew. Chem. Int. Ed.,* **1999**, *38*, 643.
2. T.A. Unger, Ed., „Pesticide Synthesis Handbook" Noyes Publications, Park Ridge, **1996**.
3. W. Herbst, K. Hunger, Eds., „Industrial Organic Pigments: Production, Properties, Applications (3rd Completely Revised Edition)" Wiley-VCH, Weinheim, **2004**.

4. A.S. Bommarius, B.R. Riebel, “Biocatalysis”, Wiley-VCH, Weinheim, **2004**.
5. U.T. Bornschauer, R.J. Kazlauskas, “Hydrolases in Organic Synthesis: Regio- and Stereoselective Biotransformations”, Wiley-VCH, Weinheim, **2006**.
6. R.J. Kazlauskas, A.N.E. Weissfloch, A.T. Rappaport, L.A. Cuccia, *J. Org. Chem.*, **1991**, 56, 2656.
7. K. Burgess, L.D. Jennings, *J. Am. Chem. Soc.*, **1991**, *113*, 6129.
8. F. Theil, *Catal. Today*, **1994**, *22*, 517.
9. A. Ghanem, H.Y. Aboul-Enein, *Chirality*, **2005**, *17*, 1.
10. N.J. Turner, *Curr. Opin. Chem. Biol.*, **2004**, *8*, 114.
11. K. Faber, “Biotransformations in Organic Chemistry”, Springer, Berlin, **2004**.
12. L. Poppe, L. Novák, “Selective Biocatalysis: A Synthetic Approach”, VCH, Weinheim-New York, **1992**.
13. A. Liese, K. Seelbach, C. Wandrey, “Industrial Biotransformations”, Wiley-VCH: Weinheim-New York, **2006**.
14. R.N. Patel, A. Banerjee, L.J. Szarka, *J. Am. Oil Chem. Soc.*, **1996***, 73,* 1363.
15. J.C. Chen, S.W. Tsai, *Biotech. Prog.*, **2000**, *16*, 986.
16. Z. Ujang, W.H. Husain, M.C. Seng, A.H.A. Rashid, *Proc. Biochem.*, **2003**, *38*, 1483.
17. P. Falus, Z. Boros, G. Hornyánszky, J. Nagy, F. Darvas, L. Ürge, L. Poppe, *Tetrahedron Lett.*, **2010**, accepted.
18. V. Bódai, R. Peredi, J. Bálint, G. Egri, L. Novák, G. Szakács, L. Poppe, *Adv. Synth. Catal.,* **2003**, *345*, 811.
19. C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, *J. Am. Chem. Soc.*, **1982**, *104*, 7294.
20. A. Tomin, D. Weiser, G. Hellner, Z. Bata, L. Corici, F. Péter, B. Koczka, L. Poppe, *Proc. Biochem.*, **2010**, doi:10.1016/j.procbio.2010.07.021
21. V. Nagy, E.R. Tőke, L. Chee Keong, G. Szatzker, D. Ibrahim, I. Che-Omar, G. Szakács, L. Poppe, *J. Mol. Catal. B: Enzym.*, **2006**, *39*, 141.